CHARACTERIZATION OF RED MUD AS A CONSTRUCTION MATERIAL USING BIOREMEDIATION

A Thesis Submitted in Partial Fulfilment of the Requirements for the Degree of Master of Technology (Research)

In

Civil Engineering

(Geotechnical engineering)

Submitted By

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2015

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DECLARATION

I hereby state that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by any other person nor substance which to a substantial extent has been accepted for the award of any other degree or diploma of the university or other institute of higher learning, except where due acknowledgement has been made in the text.

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CERTIFICATE

This is to certify that the thesis entitled **"Characterization of red mud as a construction material using bioremediation''** being submitted by Ipsita Panda in partial fulfilment of the requirements for the award of Master of Technology (Research) degree in Civil Engineering with specialization in GEOTECHNICAL ENGINEERING at National Institute of Technology Rourkela, is an authentic work carried out by her under my guidance and supervision. To the best of my knowledge, the matter embodied in this report has not been submitted to any other university/institute for the award of any degree or diploma.

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ACKNOWLEDGEMENTS

This thesis owes its existence to the help support and inspiration of several people. Foremost, I would like to express my sincere gratitude to my advisor **Prof. Sarat Kumar Das** for the continuous support of my M. Tech [R] study and research, for his patience, motivation, enthusiasm, and immense knowledge. I would like to endow my hearty regards to him for sharing his research experiences and introducing me to a new topic of research .There have been numerous occasions where I was never up to the mark, needless to say his patience in understanding and supporting me during those times cannot be forgotten. I could not have imagined having a better advisor and mentor for my M Tech [R] study.

I am also indebted to **Prof. R Jayabalan**, Department of life science for allowing me the opportunity to work and train in his laboratory, and affording me much needed guidance. As a pioneer in his field, Prof. R Jayabalan has provided me with unique insight that one would expect from such an individual.

I would like extend a special thanks to **Mr. Eldin M.J** for all his efforts including the countless hours spent providing a deeper and broader understanding of microbiology that can only come from fruitful years of academic work bolstered by equally impressive years in research field. He has been a constant source of enthusiasm not only during this project but also during the full tenure of my master's program.

I would like to take this opportunity to express special gratitude to my Parents and my family who were a constant inspiration and a source of unending cheer.

Last but not the least I would like to express special thanks to my dear friends for their unconditional love, moral support and encouragement for the completion of this research and of course would like to take this opportunity to thank all Laboratories staff of Civil Engineering and Life science Department for their help throughout my research work.

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ABSTRACT

Globally 120 million tonnes of red mud/bauxite residue is generated annually posing a very serious and alarming environmental problem. The most important barrier to remediation, re-use and long term sustainability of bauxite residue management is its high alkalinity. The main objective of the study pertains to stabilize and neutralize red mud concerning strength and stability factors viz. reclamation, wind erosion, dust control, and other harmful effects in its stacking areas. The neutralization of red mud was achieved by five different strains of microbes. Then morphological, physical and geotechnical properties of neutralized red mud are studied. Due to organic acid production by the microbes there is a noticeable change in the physical properties of the residue. Red mud is also stabilized using biopolymer Xanthan gum (1%, 2% and 3%), and a noticeable increase in unconfined compressive strength was observed for the stabilized red mud. The effect of drying and exposition to the natural environment was also studied. The results of present study will help in effective utilization of the red mud.

Keywords: Red mud; alkalinity; bioremediation; neutralization; stabilization; strength

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Chapter 1

Introduction

1.1 Introduction

Red mud is a perilous waste generated in the Bayer process of alumina production (Al_2O_3) from bauxite ore which contains high levels of residual alkalinity and toxic heavy metals viz. silica, aluminum, iron, calcium, titanium, as well as an array of minor constituents, namely: Na, K, Cr, V, Ni, Ba, Cu, Mn, Pb, Zn (Summers et al., 1996). They occupy a vast area of land which does not support any kind of vegetation because of their hostile nature vegetation growth. Because of the huge volume of the red mud being generated as a byproduct from the alumina industry it makes them nearly difficult to contain them and thus causes serious disposal problems (Gräfe and Klauber, 2011). Therefore development and implementing its storage and its remediation programs remains essential as inventory grows approximately 120 million per annum. As a result the red mud can be easily carried away and eroded by wind and heavy downpour from the dumping site to the nearby residential areas. Contact of the red mud with skin and eyes causes severe complication and health hazards to people. They cause serious threat to the ground water by seepage and also causes various environmental hazards. Table 1.1 shows a typical composition of red mud and Table 1.2 shows chemical composition of typical Indian red mud.

Table 1.1 Typical composition of red mud

Composition	Percentage
Fe_2O_3	30-60%
Al_2O_3	10-20%
SiO_2	3-50%
Na_2O	2-10%
CaO	2-8%
TiO_2	trace-25

(Source: Red mud Project. http://www.redmud.org/Characteristics.html)

Table 1.2. Chemical composition of typical Indian red muds

Company	Al_2O_3 (%)	Fe_2O_3 (%)	SiO_2 (%)	TiO_2 (%)	Na_2O (%)	CaO (%)	LOI (%)
BALCO, Korba	18.10-21.0	35.0-37.0	6.0-6.5	17.0-19.0	5.2-5.5	1.7-2.2	11.8-14.0
HINDACO, Renukoot	17.5-19.0	35.5-36.2	7.0-8.5	16.3-14.5	5.0-6.0	3.2-4.5	10.7-12.0
HINDALCO, Muri	19.0-20.5	44.0-46.0	5.5-6.5	17.0-18.9	3.3-3.8	1.5-2.0	12.0-14.0
HINDALCO, Belgaum	17.8-20.1	44.0-47.0	7.5-8.5	8.2-10.4	3.5-4.6	1.0-3.0	10.8-14.0
MALCO, Metturdam	18.0-22.0	40.0-26.0	12.0-16.0	2.5-3.5	4.0-4.5	1.5-2.5	11.0-15.0
NALCO, Damanjodi	17.7-19.8	48.2-53.8	4.8-5.7	3.6-4.1	3.8-4.6	0.8-1.2	10.8-13.5

Source: Chaddha et al. (2007)

The various environmental trouble linked with the disposal of red mud waste includes:

- The high pH (10.00-13.00) that involves a range of safety and environmental hazards including potentials for contact of humans and wildlife with caustic liquor and mud

- Contamination of underground water due to leaching of caustic liquor and associated toxic metals.

- Requires the provision of substantial areas of land for storage and that makes it difficult to close and rehabilitate because of the need to remove large amounts of caustic liquor, both supernatant and entrained and hence requires planning and funds allocation for long-term closure.

- The surfaces of the dry stack are subject to dust lift-off and mitigation processes may be required.

- Generally requires significant engineering to eliminate potential for catastrophic failure of impoundments and consequent hazard to surrounding environment and population.

Besides the above limitations and negative impacts red mud is being used in various sectors like in metallurgical ones (iron and steel production, titania, alumina and alkali, minor constituents recovery), production of building materials (constructional brick, light weight aggregates, bricks roofing and flooring tiles, cements etc.), catalysis, ceramics (pottery, sanitary ware, special tiles and glasses, glazes, ferrites) and other miscellaneous direct uses (in waste treatment, as a filler, as a fertilizer, etc.). Reviews on this subject have been also published in various papers. However the quantity of red mud used in other sectors is limited. The toxicity of red mud is the main cause for the poor utility of bulk red mud in various sectors. Therefore, it's a critical aspect of the residue that needs to be resolved.

1.2 Use of microorganisms in geotechnical field

Modification in soil eco system can be achieved by various microorganism existing in various type of soil present. Role of these living organisms creates a huge difference in the inter soil particles.

The major factors that affect the application of microorganisms in the soil ecosystem is its identification and screening for different applications in the geotechnical field. However, from various researches it has been found that microorganisms produce exopolysaccharides that helps in soil aggregation, bioclogging, biocementation and can be helpful in stabilization, mitigation of liquefaction potential, strengthening tailing dams, binding etc. (Ivanov and Chu, 2008). In today's arena use of chemical grouts like suspension of sodium silicate, acrylates, acrylamides, and polyurethanes and microbiological grouting have been augmented to improve the physical properties of soil structure. Similarly, industrially produced water-insoluble gel-forming biopolymers of microbial origin such as Xanthan, chitosan, polyglutamic acid, sodium alginate, and polyhydroxybutyrate can also be used as grouts for soil erosion control, enclosing of bioremediation zone, and mitigating soil liquefaction (Burbank et al. 2013).

Similarly there are few organisms that survive at high pH ranging environment like *Bacillus, Micrococcus, Lactobacillus, Streptococcus, Corynebacterium, Pseudomonas, Clostidium* etc. Few organisms have already been isolated from bauxite residue intimating that there survival at high pH is possible. These bacteria are effective in producing acids like acetic acid, citric acid, lactic acid, propionic acid etc. and gives a possible method of neutralizing bauxite residue by utilizing carbon source for assessment of organic acid production. (Mussel et al. 1993).

1.3 DEFINITION OF PROBLEM:

Although various research have already been carried out in neutralization of red mud, but at the cost of other borne environmental problems. The most established methods that have been implemented till date are sea water neutralization to precipitate hydroxide, carbonate and aluminate ions with magnesium and calcium but includes covering of sea bed, destruction of associated ecosystem and further potential for release of toxic metals to marine environment with increase in turbidity of sea water. The carbon dioxide neutralization is not feasible as red mud cannot be neutralized up to the considerable limit and the rate of neutralization is not fast enough to satisfy industrial needs. Addition of brine also reduces the alkalinity to a considerable limit. Similarly, neutralization by mineral acids does not provide any acid-base buffering capacity due to influence of solid hydroxides in the residue, nor does it improve the physical properties of the material. An additional problem of red mud is its in-stabilization in the lake area where it is discharged. After it dries. mud it is been carried away by wind, air etc. causing leachate of red mud that develops contamination with other natural sources, affecting the environment and constructing other health problems due to the erosion of dry red mud.

Bioremediation is the use of microorganisms for the degradation of hazardous chemicals in soil, sediments, water, or other contaminated materials. It is well known that almost all bacteria produce exopolysaccharides under excess of carbohydrates or other water soluble sources of carbon over source of nitrogen (Wingender et al. 1999). Therefore, such food-processing wastes or sub-products as corn glucose syrup, cassava glucose syrup and molasses with C: N ratio>20 are used for industrial production of bacterial water-insoluble polysaccharides (Portilho et al. 2006).

Microbial Geotechnology is a new branch of geotechnical engineering that deals with the applications of microbiological methods to geological materials used in engineering. Biogeotechnology is a branch of geotechnical engineering that deals with the applications of biological methods to geotechnical engineering problems that are related mainly to the applications of plants or vegetative soil cover for soil erosion control and slope protection, prevention of slope failure, and reduction of water infiltration into slopes and have advantages of low investment and maintenance costs.

This research work consists of two parts. First part research work deals with the neutralization of the highly alkaline red mud by microbial action by remediating the alkalinity and toxicity of the red mud and thus making it more amiable. Neutralization of red mud to pH around 8.0 is optimal because the chemically adsorbed Na is released, alkaline buffer minerals are neutralized and toxic metals are insoluble at this pH (Hanahan et al. 2004)

The second part of this research concerns in stabilization of red mud using biopolymer Xanthan gum (produced by the gram-negative bacterium *Xanthomonas campestris* by fermenting glucose, sucrose, or other carbohydrate sources) concerning stability factors viz. piping due to dispersion, wind erosion, dust control, and other harmful effects of red mud in the lagoon area. This biopolymer is an effective stabilizer as it is used in other sectors as a thickening agent, stabilizer, or emulsifier, and combined with other gums it can act as a gelling agent. (Chen and Zhang, 2013). Development of technological applications for bauxite residue treatment could change the material discharged allowing for long term storage, remediation and other re-use options. Thus the project will enable the development of best practice residue management options to reduce the reliance on stockpiling and storage, or to make stockpiling and storage more environmentally acceptable.

1.4 SCOPE AND OBJECTIVE OF RESEARCH WORK

1.4.1 OBJECTIVE OF RESEARCH WORK

To employ various microbes for the neutralization of red mud and using a naturally occurring biopolymer that will help in effective utilization of red mud as a fill and embankment material.

1.4.2 SCOPE

- Alkalinity neutralization of the red mud using bioremediation technique by employing five microbes viz. *Lactobacillus plantarum* (NCIM 2083) *Lactobacillus acidophilus* (NCIM 2660), two red mud isolates (microbes that have been isolated from red mud) and SAE 13.
- Study of physical and geotechnical properties of red mud before and after bioremediation.
- Effect of biopolymer on compaction behavior, unconfined compressive strength and dispersion behavior.

1.5 THESIS OUTLINE

Thesis is presented in 6 chapters, a brief description of red mud, neutralization and stabilization of red mud is described in Chapter 1 as Introduction. Different bioremediation procedure, neutralization methods, stabilization methods and geotechnical experiments on red med which have been done by several researchers is presented in chapter 2 as literature review.

The different materials used in the project work are described in Chapter 3. The methods applied for bio-neutralization, chemical and geotechnical properties of samples are given in this chapter.

Chapter 4 describes the results from the methods used for bio-neutralization, chemical properties of red mud, geotechnical properties of red mud and bio-neutralized red mud. It discusses about the comparison of both red mud and neutralized red mud. Similarly, Chapter 5 describes the results from stabilization of red mud using a biopolymer. Finally in Chapter 6 conclusions drawn from various studies made in this thesis are presented and the scope for the future work is presented. The general layout of the thesis work based on each chapter is shown in a flow diagram below in Figure 1.1.

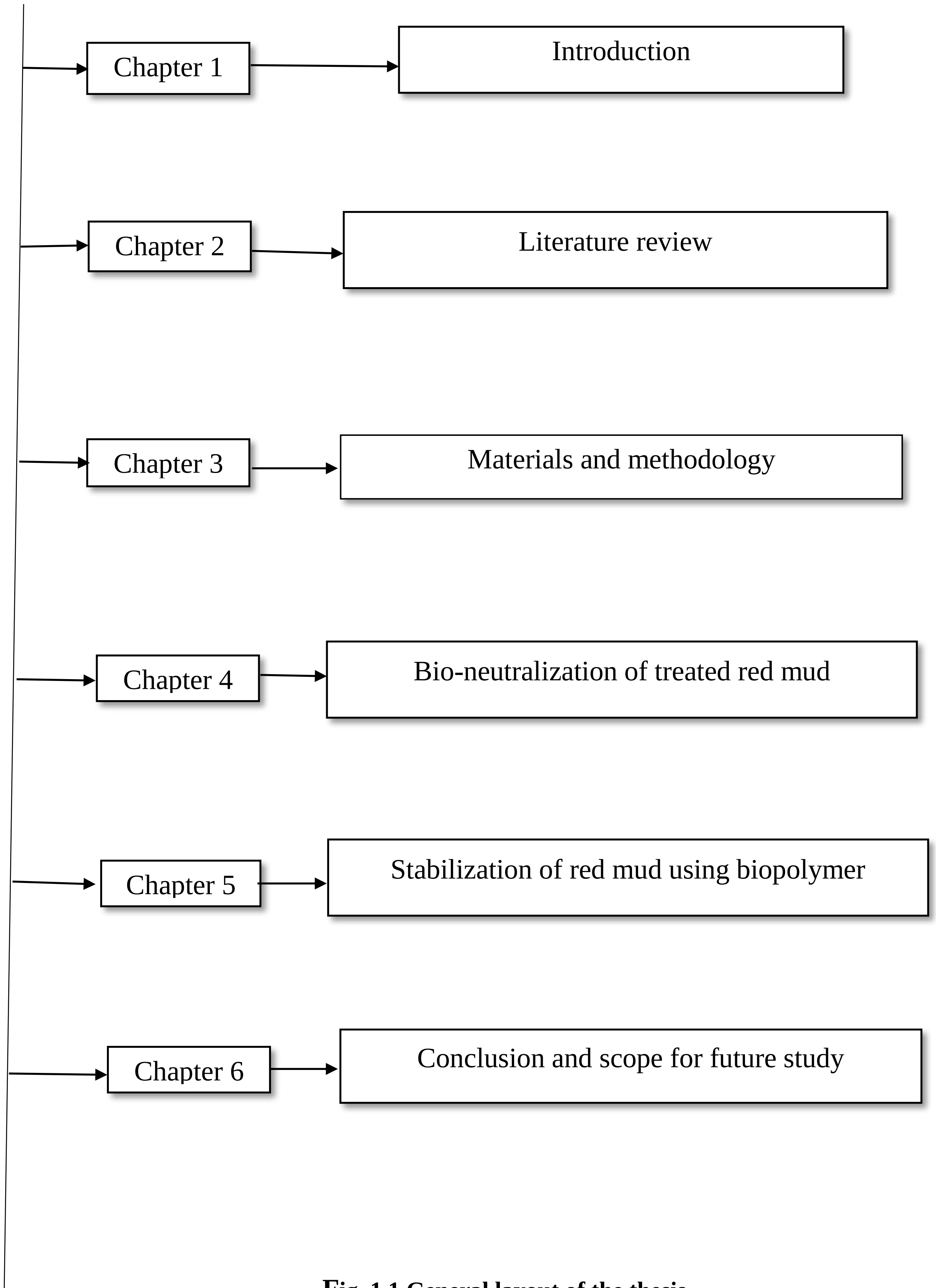

Fig. 1.1 General layout of the thesis

Chapter 2

Literature review

2.1 Introduction

This chapter discussed about the literature review for red mud. In this some studies related to characterization of red mud as construction material, neutralization of red mud, stabilization of red mud are discussed. Since last two decades various efforts have been made towards characterization and utilization of red mud and few attempts have been made for geotechnical characterization of red mud. It is also observed that few efforts have been made towards the bio-neutralization of red mud for reducing the alkalinity of red mud but no attempts have been made in geotechnical characterization of modified/neutralized red mud. This chapter discusses about the different investigation for characteristics of red mud as construction material, effective bioremediation and stabilization of red mud in different applications in general.

2.2 *Literature review on applications of red mud as construction material*

Kalkan (2006) examined the effects of red mud on the unconfined compressive strength, hydraulic conductivity, and swelling percentage of compacted clay liners as a hydraulic barrier. The test results showed that compacted clay samples containing red mud and cement–red mud additives had a high compressive strength and decreases the hydraulic conductivity and swelling percentage as compared to natural clay samples. The addition of these additives changed the soil groups from high-plasticity soil group (CH) to low-plasticity soil group (MH). Consequently, it was concluded that red mud and cement–red mud materials can be successfully used for the stabilization of clay liners in geotechnical applications.

Desai and Herkal (2010) effectively utilized red mud in making burnt and unburnt bricks in pressed and unpressed conditions. Red mud bricks with additives like lime, sand, recron fibers of 6mm &12mm length were prepared and tested. Unpressed bricks with recron fibers (6mm & 12mm) give good strength but just pressing the bricks to proctor density, almost doubles the strength. Based on the optimum percentage of fiber and cost, 12mm recron fibers can be cost effective. Burning of red mud bricks do not show any improvement in the strength. Therefore, unburnt pressed red mud bricks with 10 percent of sand or with 12mm recron fibers can be effectively used for low cost houses and interior infilled walls.

Ribeiro et al. (2011) studied on non-calcined red mud which requires less energy and time and reducing costs, which is the ideal condition for reusing wastes. In this research mortars containing 30 wt. (%) of cement substituted by red mud showed higher strength of hardened products. The pozzolanic activity index was evaluated based on physical and mechanical parameters (Brazilian NBR 5751 and NBR 5752 standards) and on a chemical analysis (European EN 196-5 standard). Then a comparison of the reference mixture (without red mud) and the results obtained with red mud confirms the potential of non-calcined red mud for use a as pozzolanic additive in cementitious materials.

Khan et al. (2012) utilized the waste from alumina refinery plant such as red mud in production of ceramic tiles. Pyrophyllite mineral had been added to the red mud to improve the strength properties. The tiles are produced at lower temperature (950-1000^{o}C) then the conventional process of making ceramic tiles and without addition of phosphatic binders. The structural features of red mud had been studied using scanning electron microscope which provides reinforcement to the ceramic tiles matrix.

Satayanarayana et al. (2012) studied red mud stabilized with 2, 4, 6, 8,10 and 12 percentages of lime and unconfined compressive strength, Split tensile strength and California bearing ratio tests were conducted at 1, 3, 7 and 28 days curing periods respectively. From the experimental findings it was observed that 10% lime has shown higher values compared to other percentages. At 28 days it has shown maximum values than other curing periods for all percentages of lime. The CBR value obtained for 10% lime at 28 days is 25%, hence it can be used as subgrade and sub base material in road construction.

Wang and Liu (2012) studied compositions and XRD patterns of the two kinds of red mud and showed that $CaCO_3$ content in Sintering red mud is significantly higher than in Bayer red mud. So it will be more applicable in the production of cement. The micro particle of Bayer red mud was finer and more disperse, but the Sintering red mud has higher shear strength. Combining the TG and hydraulic characteristics analysis, it can be shown that Bayer red mud has higher value of water content and Sintering red mud has higher hydraulic conductivity. The paper then illustrated that Sintering red mud can become the main filling material of supporting structure of red mud stocking yard. Bayer red mud has a high reuse value and also can be used as a mixing material of masonry mortar.

Rout et al. (2012) designed high embankment using red mud based on the laboratory geotechnical investigation and the stability analysis using finite element analysis. To protect the dispersive red mud against external weathering it was recommended to cover it with local soil. The embankment with soil cover was found to have more than required FOS value. The embankments were also analyzed considering concentrated load and the load with vibration. The FOS is found to decrease with static and vibration load.

Arhin et al. (2013) studied the applicability of red mud in the ceramic brick construction industry as a means of recycling the bauxite waste. The red mud-clay composites have been formulated as 80%-20%, 70%-30%, 60%-40%, 50%-50% and fired at sintering temperatures of 800ºC, 900ºC and 1100ºC. They found that physical properties such as apparent porosity and water of absorption reduced while the mechanical strength (modulus of rupture), and the bulk densities increased at higher sintering temperature.

Rathod et al. (2013) Investigated the possibility of replacing the Portland cement by red mud. In this study portland cement was replaced up to 40 % RM by wt of cement. They examined the effects of red mud on the properties of hardened concrete. The test results showed that its compressive strength & splitting tensile strength decreased with increase red mud content and concluded that Optimum percentage of the replacement of cement by weight was found to be 25%.

Satyanarayana et al. (2013) discussed use of red mud bentonite mixture as clay liner. They observed that when the percentage of Bentonite increased, consistency properties such as liquid limit, plastic limit and plasticity index are increasing. It was also observed that as bentonite increases and the red soils bentonite mixes exhibit good strength at higher percentages of bentonite and the soil bentonite mixes become impervious (k $<10^{-6}$ cm/sec). Hence from the test data it was identified that a dosage of 10-15 percent Bentonite yields satisfactory results for the use of these mixes as liner materials.

2.3 *Literature review on different method adopted for neutralization of red mud*

Paradis et al. (2007) investigated that neutral pH conditions were maintained over the entire test for red mud and a mixture of red mud with brine. Addition of brine to red mud bauxite slightly lowered the pH compared to red mud alone. Red mud alone lost a lot of dissolved alkalinity at the beginning of the test. Most of the alkalinity was lost in water after a few flushes for red mud bauxite samples. Neutral pH conditions were maintained all through the test for red mud and red mud with brine mixed with mine tailings. The shallow 0–15 cm sample, or oxidized tailings, had an initial pH 3.94 which was lower than the deep 15–35 cm sample, or unoxidized tailings (pH 6.2). Addition of 10% mass of RMB to tailings brought stable pH at values near 8 for the shallow 0–15 cm sample and 10 for the deep 15–35 cm samples. For the deep 15–35 cm unoxidized samples, where acidification potential was still present, the use of RMB treated with brine may provide a long-term alkalinity reserve to neutralize future acidification of tailings. The addition of brine helped to keep neutralization potential over more cycles of leaching.

Khaitan et al. (2009) discussed the extent of neutralization of bauxite residue by carbon dioxide as a function of CO_2 partial pressure and determined the geochemical reactions responsible for carbon sequestration. Bauxite residue was exposed to carbon dioxide (CO_2) at partial pressures ranging from 10−3.5 to 1 atm and the residue pore-water pH of 7.7 was measured until a steady state pH was achieved.

Palmer et al. (2010) investigated that when seawater was added to caustic red mud, the pH of the mixture reduced causing hydroxide, carbonate or hydroxycarbonate minerals to be precipitated. Thermally activated seawater neutralized red mud removes at least twice the concentration of anionic species than thermally activated red mud alone, due to the formation of 40–60% Bayer hydrotalcite during the neutralization process

Rai et al. (2013) studied the feasibility of using seawater to neutralize alkaline red mud for its safe disposal using Taguchi's design of experimental methodology. Parameters such as weight of red mud, volume of seawater, stirring time and temperature were tested at three levels to study their effect on response characteristic, i.e., pH of the neutralized slurry. The analysis of variance showed that volume of seawater added and quantity of red mud are the two significant parameters with 53.59 and 44.92 % contribution each, respectively. Under the optimized parameters i.e 25 g of red mud, 150 mL of seawater (i.e., solid to liquid ratio of 1:6), 30 min stirring time and 30^{o}C temperature to get a pH value of red mud slurry that reaches to about 8.0 which is within disposable limits. Weight of red mud and volume of seawater was found to be the most significant factors affecting the neutralization process with nearly equal percentage contribution. It was also observed that when seawater or other Ca- and Mg-rich brines were added to caustic red mud, the pH of the mixture was reduced causing hydroxide, carbonate or hydroxy carbonate minerals was precipitated.

2.4 *Literature review in bioremediation of various soils.*

Lovely et al. (1994) studied that microbial metal reduction has the potential to be a useful technique for the biorestoration of environments contaminated with organics and/or metals. As recently reviewed microorganisms may enzymatically reduce other metals such as vanadium, molybdenum, copper, gold, and silver.

Dejong et al. (2006) studied the results of a study in which natural microbial biological processes were used to engineer a cemented soil matrix within initially loose, collapsible sand. Microbially induced calcite precipitation (MICP) was achieved using the microorganism *Bacillus pasteurii*, an aerobic bacterium pervasive in natural soil deposits. The microbes were introduced to the sand specimens in a liquid growth medium amended with urea and a dissolved calcium source. The results of both MICP and gypsum-cemented specimens were assessed nondestructively by measuring the shear wave velocity with bender elements. A series of isotropically consolidated undrained compression (CIUC) triaxial tests indicated that the MICP-treated specimens exhibit a non-collapse strain softening shear behavior, with a higher initial shear stiffness and ultimate shear capacity than untreated loose specimens.

Ivanov and Chu (2008) concluded the majority of the studies on Microbial Geotechnology at present are at the laboratory stage. Due to the complexity, the applications of microbial geotechnology would require an integration of microbiology, ecology, geochemistry, and geotechnical engineering knowledge. The aim of these applications was to improve the mechanical properties of soil so that it would be more suitable for construction or environmental purposes. Two notable applications, bioclogging and biocementation, had been explored.

Natarajan et.al (2008) studied various types of *Acidithiobacillus* group of bacteria which are responsible for acid mine drainage were isolated from different Indian mines. Native bacteria such as *Thiomonas* and *Bacillus* spp. exhibited higher arsenic tolerance and were capable of oxidizing arsenite to arsenate. Biological methods of remediation of acid mine drainage and bio removal of copper, zinc, iron and arsenicare illustrated. Sulphate Reducing Prokaryotes could effectively precipitate all the above dissolved species as sulfides.

Kavazanjian Jr et al. (2009) investigated the application of biopolymers (Xanthan gum and chitosan gum) as soil stabilizers to mitigate wind induced erosion, either by spraying biopolymer solutions at different concentrations on the surface of soil or mixing the solutions with soil prior to compaction. The results showed that both methods were effective in mitigating wind induced soil erosion. The enhanced wind erosion resistance by surface-spraying came from the formation of crust on the surface of treated soil. The mixing and compaction method could achieve similar improved resistance results but was more expensive. The effectiveness of the biopolymer treatment lasted for at least two weeks when exposed to sunlight and summer temperature.

Dejong et al. (2010) observed that bio-induced mineralization in soils may reduce the pore space of soil and strengthen the particle contacts, leading to increased strength and decreased permeability and compressibility.

Landa and Santamarina (2012) discussed sands, silts, and clayey sands inoculated with *Paracoccus denitrificans* when monitored to assess the effects of nutrient availability, fines content, and pressure-diffusion on the evolution of nitrogen gas generation and bulk stiffness. Results show clear evidence of biogas bubble formation, earlier gas generation and entrapment in specimens with higher fines content, and a strong correlation between biogas volume and P-wave velocity. The volume of gas is correlated with specific surface, suggesting that biogas bubble formation develops as heterogeneous nucleation and that it is directly linked to the availability of nucleation sites on mineral surfaces, which in turn also affect the degree of attainable super saturation. Biogenic gas generation (nitrogen gas in this study) effectively reduces the bulk stiffness of the pore fluid, the P-wave velocity, Skempton's B parameter (relative to the soil shear stiffness), and the susceptibility to liquefaction

He et al. (2013) developed a biogas method to overcome this difficulty in injecting bacteria into soil pores. In this method, denitrifying bacteria are used to generate tiny, inert nitrogen gas bubbles in sand. Shaking table tests using a fully instrumented laminar box are conducted on both saturated sand and sand containing microbially generated nitrogen gas bubbles. Test results showed that liquefaction occurred for saturated samples at loose states under amax ¼ 0.5 m/s2 and at medium dense states under a max ¼ 1.5 m/s^2. The model tests have demonstrated that the biogas method is effective in lowering the degree of saturation and reducing significantly the liquefaction potential of saturated sand deposit.

Chen and Zhang (2013) studied Xanthan gum and guar gum, two biopolymers that are naturally occurring and inexpensive, to stabilize mine tailings (MT). They found out that guar gum is more effective than Xanthan gum in increasing the wL and su of MT, because the guar gum solution is more viscous than the Xanthan gum solution at the same concentration, the guar gum–MT particle bonding is stronger than the Xanthan gum–MT particle bonding, and guar gum causes a lower degree of aggregation of MT particles than Xanthan gum. By comparing the undrained shear strength data with empirical equations in the literature, two new equations were proposed for predicting the undrained shear strength of the MT mixed with a biopolymer for water contents near the liquid limit, based on the liquid limit and water content, and the liquidity index.

Morales et al. (2014) analyzed the possibility of stabilizing compacted soils by a soft bio-mediated treatment. The treatment consists in adding microorganism of Bacillaceae family to the compaction water content and relying on the natural availability of urea and Ca^{+2} in the superficial soils used in the construction. Adding microorganism to the soil promotes the formation of an aggregated structure, which remains after compaction. The precipitated crystals filled the pores in the range 3–50 µm, and the pore volume of the material tends to decrease as a consequence of the progressive filling of the inter-grain/inter-aggregate porosity. The change in the pore size density function was reflected consistently in the hydraulic and mechanical behavior of the treated soil, which presents typical features of a denser soil with respect to the untreated one. Filling part of the soil macro porosity of the treated samples affected the water retention properties inducing slightly higher air-entry value and lower water permeability at comparable void ratios. Treated soils displayed a slightly higher friction angle with no cohesion in the shear envelope, consistently with the pattern of a denser granular soil. The small-strain shear stiffness was increased, and collapse on wetting for a given initial void ratio reduced together with the post-yield compressibility on loading.

2.5 *Literature review of red mud used in bioremediation*.

Mussels et al (1993) did a review to assess the feasibility of bioremediation of bauxite residue by making estimates of the possible substrate and inoculums required to achieve neutrality. Various possible avenues of microbial neutralization were assessed in terms of the practicality of application of bauxite residue. These involved the ability of an organism to survive and grow in red mud and their production of neutralizing agent mainly organic acid and carbon dioxide.

Valerie Ee (1999) investigated the microbiology of red mud and ascertained the possibility of using bacteria to reduce the alkalinity of red mud. To achieve this, several properties of red mud were analyzed and bacteria were isolated from the red mud sample. Characteristics of these indigenous bacteria were investigated, including their ability to produce acidic substances in alkaline glucose medium. Finally, the ability of these acid producing bacteria to reduce the alkalinity of glucose amended red mud was determined using a series of pot trials.

Hamdy and Williams (2001) demonstrated that low levels of injured bacterial cells in the bauxite residue actively grew using various added nutrients and/or hay. The organisms grew from less than 10 to more than 109 cells g_1 bauxite residue and formed organic acids that lowered the pH from 13 to about 7.0. The pH of treated bauxite residue decreased from an initial value of about 11.0 to 6.5 for all samples after 20 days incubation (37^{o}C) under aerobic or anaerobic conditions. A total of 150 cultures was isolated from treated bauxite residue and included species of *Bacillus, Lactobacillus, Leuconostoc, Micrococcus, Staphylococcus, Pseudomonas, Flavobacterium* and *Enterobacter.* The treated bauxite residue supported growth of several plants and earthworms that survived for over 300 days. In a test plot bioremediation on a residue deposit, the Bermuda grass hay used was effective mulch material and encouraged water filtration, leading to establishment and growth of salt –tolerant vegetative species.

Krishna and Patnaik (2005) used *Aspergillus tubingensis* (AT1), a phosphate solubilizer in red mud amended soils (0, 25, 50 and 75%) to reduce the alkalinity of the red mud. *A. tubingensis* was tested for its ability to grow at high pH and in different concentrations of aluminum (Al), iron (Fe) and sodium (Na). The results showed that it was able to grow at pH values from 2.5 to 12.0. The maximum growth was observed at the pH 3.5. The growth of *A. tubingensis* was significantly inhibited by Al at 200μg/ml. The Na content increased in the mycelium as the concentration of Na increases in the growth medium and the maximum accumulation was found at 200 μg/ml .The red coloration of red mud is due to high iron (Fe3+) levels in the primary ore minerals. The ability of *A. tubingensis* to grow in presence of different Fe concentrations was tested and the results indicated that the best growth of *A. tubingensis* was achieved at concentrations of 400μg/ml. At higher Fe concentrations (500 and 600μg/ml) the growth was slightly increased when compared to control. The maximum growth was observed when the fungus was grown at 10% red mud amended medium. .*A. tubingensis* was able to grow at high pH and reduce the alkalinity of the nutrient medium and also to accumulate different metals in the mycelium.

Das and Dandapat (2011) used the red mud waste as a media for fungal growth and maintained in the form of a solution and was added to the organic solution to prepare samples of different pulp density (i.e.20%, 40%, 60% and 80% w/v). The pH for different pulp densities of red mud with the period of incubation was observed after treatment with the fungal rich organic media. The pH as a function of initial pH, concentration of red mud and incubation period was modelled using the neural networks. The indigenous fungi (*Aspergilus niger*) was used for production of organic acids results in a progressive decrease in the pH of the media which can also be associated with the fungal growth. The leaching ability of fungi is due to its acidolysis and complication phenomena. The pH as a function of initial pH, concentration of red mud and incubation period was modelled using the neural networks. The pH was observed for 30 different combinations of parameters like initial pH, concentration of red mud in media and period of incubation.

Different types of bacteria of varying degrees of coping with at high alkalinity and salinity were isolated from red mud. The experiments revealed that oxygen was necessary for pH reduction under the chosen conditions, and that the pH reduction was related to bacterial growth. They suggested the possibility to reduce the pH of red mud by bacterial metabolism in the presence of aerobic condition, by inoculating the red mud with pure cultures of indigenous bacteria, and incorporating nutrients to encourage bacterial growth.

From the studies of the available literature it is observed that various efforts have been made to study the geotechnical properties of red mud, its stabilization and its application in various sectors, but few a very researches have been made on its neutralization. A concise literature review as above is presented in Table 2.1 to Table 2.4. From various literature studies it is observed that limited study is done on neutralization of red mud using microbes.

Table 2.1 ***Literature review on applications of red mud as construction material.***

SL No	Reference	Soil or bacteria type	Investigation	Findings
1.	Kalkan (2006)	Red mud, cemented red mud	UCS, hydraulic conductivity & swelling percentage	Increases compressive strength and decreases the hydraulic conductivity and swelling percentage, soil groups changed from high-plasticity soil group (CH) to low-plasticity soil group (MH).
2.	Desai and Herkal (2010)	Red mud, lime, sand & recron fibers	Proctor density	Unpressed bricks with recron fibers had good strength and used in low cost houses and interior infilled walls.
3.	Ribeiro et al. (2011)	Red mud, Mortar, Cement	setting time, workability	The setting time (according to the MERCOSUL NM 65 standard) tends to increase but workability remains almost unchanged.
4.	Khan et al. (2012)	Red mud	Production of ceramic tiles	Pyrophyllite mineral added to the red mud to improve the strength properties.
5.	Satayanarayana et al. (2012)	Red mud, lime	UCS,CBR, split tensile strength	10% lime has shown higher values, CBR value for 10% lime at 28 days is 25% so, can be used as subgrade and sub base material in road construction.
6.	Wang and Liu (2012)	Red mud, sintering red mud	XRD, TG, SEM	Higher shear strength of Sintering red mud, higher water content of bayer red mud and higher hydraulic conductivity of Sintering red mud.
7.	Rout et al. (2012)	Red mud	FEM analysis	FOS decreases with static and vibration load.
8.	Arhin et al. (2013)	Red mud, red mud-clay composites	apparent porosity water absorption,modulus of rupture,bulk density	Apparent porosity and water of absorption reduced, mechanical strength (modulus of rupture), and bulk densities increased at higher sintering temperature.

9.	Rathod et al. (2013)	Portland cement, red mud	compressive strength & splitting tensile strength	Studied the effects of red mud on the properties of hardened concrete. compressive strength & splitting tensile strength decreased with increase red mud content.
10.	Satyanarayana, et al. (2013)	Bentonite, red mud	liquid limit, plastic limit and plasticity index	Consistency properties increases and red mud bentonite mix is more impervious.

Table 2.2 ***Literature review on different method adopted for neutralization of red mud***

SL. No	Reference	Soil type	Investigation	Findings
1.	Paradis et al. (2007)	Red mud, brine	Neutralization using brine	Gives a long-term alkalinity reserve to neutralize red mud bauxite in future acidification of tailings
2.	Khaitan et al. (2009)	Red mud	neutralization of red mud by carbon dioxide	Reduction in pH of red mud up to 7.7
3.	Palmer et al. (2010)	Red mud, sea water	Neutralization of red mud using sea water	Thermally activated seawater neutralized red mud removes twice the concentration of anionic species than thermally activated red mud alone
4.	Rai et al. (2013)	Red mud, sea water	using seawater to neutralize alkaline red mud for safe disposal	pH value of red mud slurry reaches to about 8.0 which is within disposable limits.

Table 2.3 ***Literature review in bioremediation of various soils.***

SL. No	Reference	Soil or bacteria type	Investigation	Findings
1.	Lovely et al. (1994)	Soils, vanadium, molybdenum, copper, gold, and silver.	Microbial metal reduction	Microorganisms enzymatically reduce other metals for bio restoration of environments.
2.	Dejong et al. (2006)	Sand, *Bacillus pasteurii*	CIUC test, MICP	Non -collapse strain softening shear behavior, higher initial shear stiffness and ultimate shear capacity of sand than untreated loose specimens.
3.	Ivanov and Chu (2008)	Microorganisms, natural soil	Microbial Geotechnology in bioclogging and biocementation	Application of microorganism in pore filling, particle binding reduction in hydraulic conductivity and increase in stiffness.
4.	Natarajan et.al (2008)	Mining,*Acidithio bacillus*	remediation of acid mine drainage	Bio removal of copper, zinc, iron and arsenicare from mine areas.
5.	Kavazanjian Jr et al. (2009)	Soil, biopolymers (Xanthan gum and chitosan gum)	Compaction, surface-spraying	Mitigating wind induced soil erosion.
6.	Dejong et al. (2010)	Soils	bio-induced mineralization	Reduction in pore space of soil and strengthen the particle contacts, increased strength and decreased permeability and compressibility.
7.	Landa and Santamarina (2012)	sands, silts, and clayey sands,	biogas bubble formation, bulk stiffness	Reduction in the bulk stiffness of the pore fluid, the P-wave velocity, Skempton’s B parameter (relative to the soil shear stiffness), and the susceptibility to liquefaction

		Paracoccus denitrificans		
8.	He et al. (2013)	Sand , denitrifying bacteria	Biogas, Degree of saturation	Lowering the degree of saturation and reducing the liquefaction potential of saturated sand deposit.
9.	Chen and Zhang(2013)	Mine tailings, Xanthan gum, guar gum	Liquid limit, water content, undrained shear strength , stabilization	Liquid limit and undrained shear strength increases on use of biopolymer. Guar gum–MT particle bonding is stronger than the Xanthan gum–MT particle bonding, & causes a lower degree of aggregation of MT particles than Xanthan gum.
10.	Morales et al. (2014)	Sand , microorganism (bacillaceae family)	Compaction, hydraulic and mechanical behavior	Higher friction angle with no cohesion in the shear envelope. Increase in small-strain shear stiffness, lower water permeability.

Table 2.4 ***Literature review of red mud used in bioremediation***.

SL. No	Reference	Soil or bacteria type	Investigation	Findings
1.	Mussels et al (1993)	Red mud	bioremediation	Ability of few organisms to grow in red mud to achieve neutrality by producing organic acid and carbon dioxide.
2.	Valerie Ee (1999)	Red mud	Microbiology of red mud	acid producing bacteria reduces the alkalinity of glucose amended red mud

3.	Hamdy and Williams (2001)	Red mud, hay ,Bacteria like *Bacillus, Lactobacillus, Leuconostoc, Micrococcus, Staphylococcus, Pseudomonas, Flavobacterium* and *Enterobacter*	Neutralization	pH reduces from 11.0 to 6.5, supported plant growth.
4.	Krishna and Patnaik (2005)	Red mud, *Aspergillus tubingensis*	Neutralization	Reduction in alkalinity of red mud
5.	Das and Dandapat (2011)	red mud, *Aspergilus niger*	pH	Reduction of pH by bacterial metabolism in aerobic condition.

Chapter 3

Materials and methodology

3.1 Introduction

This chapter discusses about the materials used and the methodology followed in the present research. The research work comprises of experimental procedure for bioremediation and stabilization of red mud and characterization and comparison of red mud before and after bio-neutralization and stabilization. The experimental methods refers to neutralization of the red mud by microorganisms and further characterizing the bio-neutralized red mud in terms of chemical, mineralogical and geotechnical properties and compare it with the raw red mud. The second part consists of experimental methods to stabilize the red mud using a biopolymer. A brief introduction about the above materials and methodology is presented as follows.

3.2 MATERIALS

3.2.1 Red mud

Red mud (RM) was collected from NALCO, Damanjodi, Odisha. About 1.5 tons of red mud is coming out per ton of alumina produced from the above plant and is discharged in a slurry form to red mud pond, which is about 212 hectares. The slurry has a composition of 45% liquid and 55% solids. Output of red mud is 200 t/hour (solids). There is about 20 million tons of waste products accumulated. Fig shows the discharge of red mud as slurry to the red mud pond and the lake view at NALCO, Damanjodi, Odisha. Figure 3.1 and 3.2 shows the discharge of red mud as slurry to the red mud pond and red mud pond at NALCO, Damanjodi. Figure 3.3 shows red mud used in present experimental work.

Fig 3.1 Discharge of red mud as slurry to the red mud pond

Fig 3.2 Red mud pond at NALCO, Damanjodi

Fig. 3.3 Collected red mud used for various laboratory purpose

3.2.2 Cheese whey

Cheese whey (CW) is a dairy product made of whey, the by-product of cheese making and has been used in several commercial uses. After the production of most cheeses, about 50% of milk solids remain in the whey, including most of the lactose and lactalbumin. Cheese whey was collected from markets as they are regarded as a waste for the food industry. Figure 3.4 shows cheese whey collected from the nearby market to be used as a part of neutralization.

Fig. **3.4** Cheese whey

3.2.3 Sugar molasses:

Molasses (SM) is otherwise known as black treacle is a viscous by-product of the refining of sugarcane into sugar and are rich in iron content. They act as a carbon source for bacterial utilization of sucrose content. Sugar molasses was collected from M/s Dharani Sugar Industries, Nayagarh, Odisha. Figure 3.5 shows the molasses collected from a sugar industry to be used in experimental works.

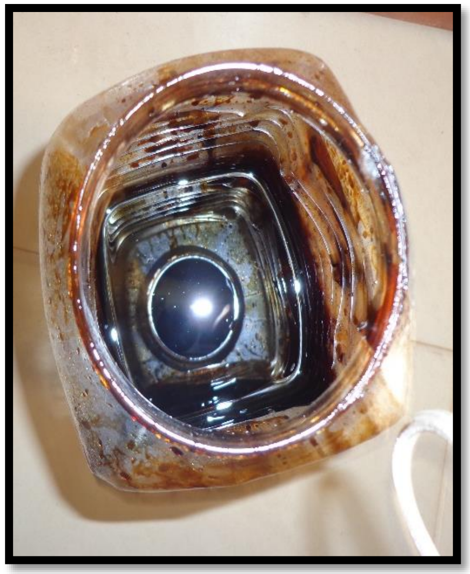

Fig. 3.5 Sugar molasess

3.2.4 Rice water

Rice water (RW) is the suspension of starch obtained by draining boiled rice or by boiling rice until it completely dissolves into the water. It considered as a waste as it is thrown away after boiling of rice. In the present study rice water is used as source of starch for bacterial growth. Figure 3.6 shows rice water collected from CVR Hall of residence mess at NIT Rourkela to be used in experimental works.

Fig.3.6 Rice water

3.2.5 Xanthan gum: The biopolymer, Xanthan gum (XG) was purchased from a Loba Chemie company. This biopolymer was chosen, because they are commercially available at relatively low prices compared with other types of biopolymers and extensive literature is available in its physical properties, and studies have shown that they are effective in stabilizing soils and have outstanding characteristics of metal chelation (Kim et al. 2009) and also they have already been found to be effective for stabilization of mine tailings (Chen and Zhang 2013). It is composed of pentasaccharide repeat units, comprising glucose, mannose, and glucuronic acid in the molar ratio 2.0:2.0:1.0 (Chen and Zhang 2013).

3.2.6 Microorganisms

Lactobacillus plantarum **(NCIM 2083) (LAB 2)** and ***Lactobacillus acidophilus*** **(NCIM 2660) (LAB 4)** were collected from National Centre for Industrial Microorganisms, National Chemical Laboratory, Pune and grown in MRS broth and were sub cultured periodically. Similarly two species were isolated from red mud itself (**RM 1C** and **RM 2A**) which are found to be acidic in nature as they are capable of reducing the pH of media. Similarly **SAE 13** and **SAN 10** belonging to Pediococcus species isolated from feces of Rourkela's healthy people by Life Science department, NIT Rourkela were also implemented. Three ***Xanthomonas campestris*** species **(NCIM 2956, NCIM 2961, and NCIM 5028)** collected from National Chemical Laboratory, Pune was also used.

3.3 METHODS

The research work consists of experimental procedure for bioremediation of red mud and characterization and comparison of red mud before and after bio-neutralization. The experimental methods refers to neutralize the red mud by microorganisms, characterize the bio-neutralized red mud in terms of chemical, mineralogical and geotechnical properties and compare it with the original red mud. Another part consists of experimental studies on the use of biopolymer for the stabilization of red mud. The experimental methods adopted in the present study are elaborated as follows.

3.3.1 Bioremediation method

MGYP media

The media so named consists of malt Extract broth base, glucose anhydrous, yeast extract powder and peptone powder. The liquid media is known as broth and the solid media is known as agar. Preparation of media is done by adding requisite amount of each powder to distilled water and mixing it. pH of media is around 6.6-6.8. After preparation of media it was plugged tightly and wrapped. Then it was autoclaved at 121^{o}C (15lbs) pressure for 20 minutes. This media is used as Malt Extract Broth Base provides carbon and nitrogen source, Glucose Anhydrous provides carbon source, Peptone Powder are a good source of nitrogen. Similarly, Yeast Extract Powder has nitrogenous compounds, carbon, sulfur, trace nutrients, vitamin B complex and other important growth factors, which are essential for the growth of diverse microorganisms. The cultures used throughout the experiment excluding lactobacillus species were sub cultured in MGYP media every 15 days.

MRS Agar

The media is so-named by its inventors: de Man, Rogosaand Sharpe. MRS agar typically contains(w/v)- 1.0 % peptone, egg extract of 0.8 %, 0.4 % yeast extract, 2.0 % glucose,0.5 % sodium acetate trihydrate, 0.1 % polysorbate, 0.2 % dipotassium hydrogen phosphate, 0.2 % triammonium citrate, 0.02 % magnesium sulfate heptahydrate, 0.005 % manganese sulfate tetrahydrate, 1.0% agar, and pH adjusted to 6.2 at 25°C (source: https://en.wikipedia.org/wiki/MRS_agar). This media is formulated for abundant growth of lactose fermenting bacteria. No pathogenic bacteria can survive in this specialized media. All the lactobacillus species used in this experiment are sub cultured in MRS media every 15 days.

3.3.1.1 Isolation of microorganisms from red mud (serial dilution process)

The standard plate count is a reliable method for enumerating bacteria and fungi. A set of serial dilutions is made, a sample of each is placed into a liquefied agar medium, and the medium poured into a petri dish. MGYP agar was prepared and autoclaved. After cooling it was poured in the petri plates for solidification. Each diluted sample was spread in to the solid medium by L-shaped bent loop from higher concentration to lower concentration (10^{-5}–10^{-1}) as shown in the figure below. The levelled petri plates are incubated at 30^{o}c for 48 hours in B.O.D incubator for growth of microorganisms. This method is known as spreading plate method. The levelled petri plates are incubated at 30^{o}C for 48 hours in B.O.D incubator for growth of microorganisms. Figure 3.7 shows the serial dilution method.

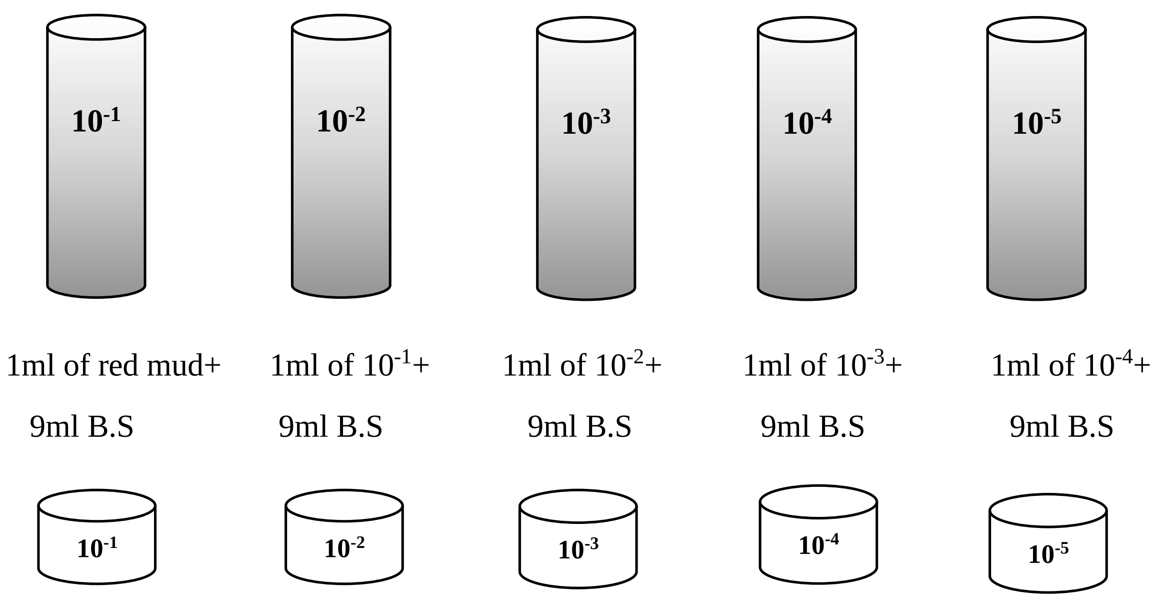

Fig. 3.7 Serial dilution method

After 48 hours it was observed that 10^{-1} plate has densely packed colonies and 10^{-5} plate has loosely packed four different colonies. The four different colonies were picked up and was sub cultured preparing the agar media by L spreader as shown in Figure 3.8. Figures 3.9 and 3.10 show the image of autoclave and B.O.D incubator respectively, used throughout the experiment.

Fig. 3.8 L shaped bent loop

Fig. 3.9 Autoclave

Fig. 3.10 B.O.D incubator

3.3.1.2 Streak plate method

Streak plate method is used for obtaining pure culture from colonies formed in petri plates. Four colonies that was sub cultured was further picked up and streaking was done to identify the grown up colonies. MGYP agar media was prepared, autoclaved, cooled and taken inside the sterilized laminar flow. The streak plate method refers to four-quadrant streak pattern which is used with an inoculation loop. In streaking all strokes are done in same direction and not multi directional as shown in Fig. The inoculation loop was heated up to red mud for sterilization after every stroke. The nichrome wire of loop are flamed to burn up any organisms and stroked through first quadrant when cooled. Microorganism culture was again stroked in the second quadrant in the same procedure. The loop is heated after the second strike and cooled. Similarly third quadrant and fourth quadrant is streaked in a similar manner. Fig 3.11 shows streak plate method and 3.12 shows inoculation loop used in the experiment. It was named as RM 1, RM 2, RM 3, and RM 4. Similarly it was observed that RM 1 had four different colored and different sized colonies, RM 2 had two different colonies, RM 3 had two different colonies and RM 4 had a single colony formed. Streaking was done again and nine more colonies were got. They were streaked again and pure colonies were obtained.

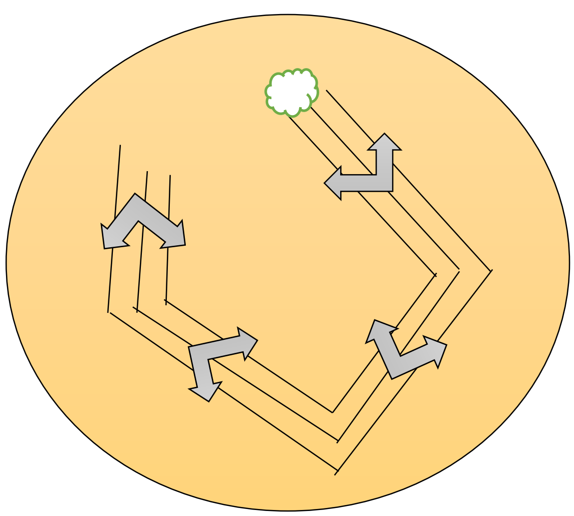

Fig. 3.11 Streak plate method

Fig. 3.12 Inoculation loop

After streaking spread plate method was again repeated for each species so that a single colony can be picked up for each. Few hundred grams of red mud added to MGYP media was taken and autoclaved .Then it was transferred to falcon tubes and each bacteria streaked was inoculated along with two probiotic bacteria (SAN 10 and SAE 13). The tubes are always taken in duplicates. After 5 days it was observed that RM 1C, RM 2A, X camp 2956 and SAN 10 reduced the pH of red mud to less than 7.5.

Centrifugation

A centrifuge is an equipment that puts an object in rotation around a fixed axis (spins it in a circle), applying a potentially strong force perpendicular to the axis of spin (outward). The centrifuge works using the sedimentation principle, where the centripetal acceleration causes denser substances and particles to move outward in the radial direction. At the same time, objects that are less dense are displaced and move to the center. In a laboratory centrifuge that uses sample tubes, the radial acceleration causes denser particles to settle to the bottom of the tube, while low-density substances rise to the top. Here centrifuge is done to get pellets of bacterial culture from MGYP media. Due to the above mechanism the pellets grown in media settles down leaving the supernatant at the top. The pellets were collected and stored at -80^oC so that it can be used in future. Fig 3.13 shows the centrifuge used for collection of pellets

Fig. 3.13 Centrifuge model 5430 R

3.3.1.3 Nursery Trials

After identification of fastest pH reducing colonies grown in MGYP media, a cheap media was again formulated using resources like rice water, cheese whey along with few chemicals like dipotassium phosphate (K_2HPO_4), diammonium phosphate $(NH_4)_2SO_4$, magnesium sulphate ($MgSO_4$) and ammonium citrate ($C_6H_{17}N_3O_7$). Then the initial pH was adjusted to ~7 by adding Sodium Hydroxide Pellets. 30% of red mud was added to it and all the pH reducing bacteria including four lactobacillus strains was also inoculated and kept in B.O.D incubator for 5 days and pH was checked again after 5 days. It was found that all the pH reducing bacteria could not utilize the carbon source present in the formulated media and couldn't grow due to lack of some of its growth source. Again the media was formulated using sugar molasses, cheese whey, rice water and few chemicals in a trace amount like dipotassium phosphate (K_2HPO_4), ammonium citrate ($C_6H_{17}N_3O_7$), calcium carbonate ($CaCO_3$), and magnesium sulphate heptahydrate ($MgSO_4.7H_20$)

and the initial pH was adjusted to ~7 by adding Sodium Hydroxide Pellets. As previously bacteria could not grow in 30% of red mud, again 15% red mud, 30% of red mud and only molasses media was taken and all the pH reducing strains along with lactobacillus species was added to each tube in the sterilized laminar flow and kept in B.O.D incubator for 7 days and pH was checked again after 7 days. It was observed that bacteria couldn't grow in red mud with molasses media but it utilized the carbon sources present in sugar molasses and reduced the pH of only molasses media. To counter check same process was repeated twice and results showed that bacteria could only utilize the molasses media and reduced the pH. Then red mud was added in various percentages (5%, 10%, 15%, 20%, 25%, and 30%) to the media with reduced pH. It was noticed that up to addition of 15% of red mud the pH is remaining neutral but when 20% of red mud was added pH increases to above 8.

3.3.1.4 Bio-neutralization process

Fermentation Medium

After nursery trials following media was formulated and all the pH reducing strains were inoculated. For pH reduction following constituents were used. Table 3.1 shows the constituents used in microbial treatment.

Table 3.1 Constituents for microbial treatment

Constituents	Amount
Sugar molasses	4% w/v
Cheese whey	10% w/v
Rice water	5% w/v
Dipotassium phosphate	3g/l
Magnesium Sulphate heptahydrate	0.25g/l
Calcium Carbonate	1g/l
Ammonium Citrate	2g/l

The above chemicals were added with 85% distilled water of known pH. Then the initial pH was adjusted to ~7 by adding Sodium Hydroxide Pellets.

Method

The media was autoclaved at 121ºC at 15lbs pressure for 20 minutes and was allowed to cool down after autoclaving. After cooling, it was taken inside the laminar flow and all the pH reducing bacterial cultures were added to it. The media was then kept in the B.O.D incubator shaker @110 r.p.m for 8-10 days. The pH was checked again after a week and was found to ~ 5 for each strain as they have utilized the carbohydrate content present in media and produced acid in it. 15 % of red mud was added to each of the reduced pH media flask and kept overnight for shaking. After 24 hours, the media was taken out and pH was checked once again. The pH was found out to be around 7.5 which is optimal. This treated red mud was kept for drying. After drying pH was again checked with distilled water with a known pH and then other geotechnical properties was studied.

3.3.1.5 Staining procedure

Staining procedure is used to study the morphology of microorganism culture. Staining techniques used to improve contrast in the microscopic image and to check whether it is gram positive or gram negative in laboratory. Stains and dyes are frequently used in microbiology for highlighting structures in bacterial culture for viewing, often with the help of different microscopes. The procedure for staining of the two isolated bacterial culture used for pH reduction i.e. RM 1C and RM 2A is described in this section.

The dried and cleaned micro slide was cleaned with ethanol. The inoculation loop was flamed for red hot. The entire nichrome wire was heated to red hot for sterilization. After cooling, a loop of sample from broth media (MGYP+ bacteria) was taken and spread over an area in micro slide. The smear was allowed to dry and heat fixing was done. Following the procedure in the gram staining kit firstly the smear was stained by crystal violet for one minute. The slide was then rinsed off with tap water to wash out the excess stain. The back of slide was wiped by paper towel and dried. Then Gram's iodine was spread and kept for one minute and washed and dried. Then decolorizer and safranin was added respectively following the same procedure. After the smear was dried, it was placed on the microscope stage and focused using the 10X, 40X objective. The smear can be focused with the 100X objective by applying oil. The gram staining procedure is shown in the shown in Figure 3.14.

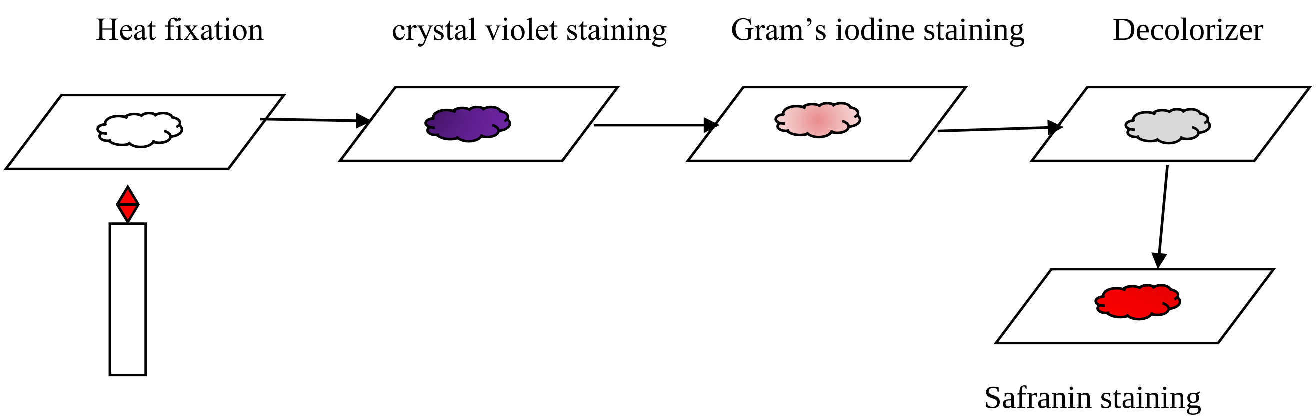

Fig. 3.14 Gram staining method

3.3.1.6 Total carbohydrate estimation

The anthrone reaction procedure is the most suitable and reliable method to calculate total carbohydrate contain in free or in any solution. Due to the use of sulphuric acid carbohydrates get hydrolyzed in simple sugars. Glucose gets dehydrated to hydroxyl methyl furfuralin, the environment of hot acidic medium. Here sugar molasses media was prepared and all pH reducing strains were inoculated and kept in incubator for 10 days to check the utilization of carbohydrates by bacteria. In this procedure about 100 ml of concentrated sulphuric acid was put in freezer before

24 hours of experiment. About 0.2g of anthrone was added in the closed container and mixed carefully in magnetic stirrer for about 20-25 minutes. The solution was then chilled and added to test tubes containing the 1st and 10th day diluted samples of five pH reducing strains and sugar molasses media, cheese whey, sugar molasses and rice water. Similarly blank solution and stock solutions of known concentrations were also made and kept in water bath at 100^{o}C for about 15 minutes. The solution formed a green colored solution on reaction with anthrone. The test tubes were kept for cooling and then absorbance values were recorded with an absorption at 620nm for all the samples. Calibration curve was prepared from absorbance readings of standards and total carbohydrate of the samples were calculated. Figure 3.15 and Figure 3.16 show the magnetic stirrer and water bath respectively, used in the experiment.

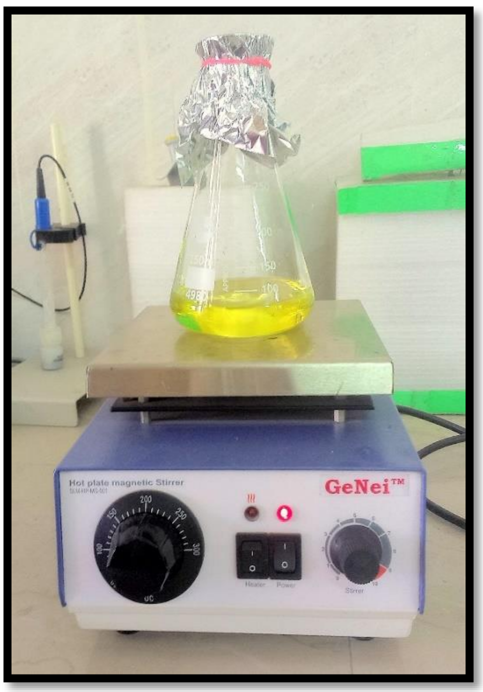

Fig. 3.15 Magnetic stirrer

Fig 3.16 Water bath

Spectrophotometer

The instrument used in recording the absorbance of samples is shown in Figure 3.17. This instrument is used for measuring the quantity of light of a particular wavelength which passes through a medium. The instrument is based on the principle of Beer's law which states that the amount of light absorbed by a medium is proportional to the concentration of the absorbing solution used. As the anthrone solution becomes green colored and green has a wavelength of 495-570nm. Concentration of a colored solution may be determined by measuring the absorbance of light at a given wavelength in the laboratory. Hence 620nm was used to determine the absorbance values of samples. The cuvette used should be washed with distill dilute water first and used carefully as per instructions. Care was taken that the cuvette was not scratched and was clean after every use to get a proper absorbance value. Figure 3.17 shows the spectrophotometer used in the experiment.

Fig. 3.17 Spectrophotometer

3.3.1.7 Study of geotechnical properties

Some of the geotechnical properties on red mud are particle size distribution, specific gravity, liquid limit, plastic limit, dry density, optimum moisture content, unconfined compressive strength etc. All the geotechnical properties of red mud have been found as per IS: 2720. The pH values are found out by Electronic pH meter and conducted as per SP: 36 (Part 1).

Determination of pH value

The pH values was found out by electrometric pH meter by means of an electrode assembly consisting of one glass electrode and one calomel reference electrode with a saturated potassium chloride solution. Potassium chloride is used for salt bridge because of the fact that the transference of the K+ and Cl- ions takes place at the rate in true solution. In this experiment buffer solutions of pH 7.0 (at 25ºC) dissolve 5.106 g of potassium hydrogen phthalate in distilled water and dilute to 500 ml with distilled water. Then 30 g of the sample was taken as prepared as per IS: 2720 (Part - 1) (1983) in a 100-ml beaker with 75 ml of distilled water and stirred for a few seconds following as per SP 36 (Part - I) of IS: 2720 (Part - 26) (1987). Figure 3.18 shows the image of pH meter.

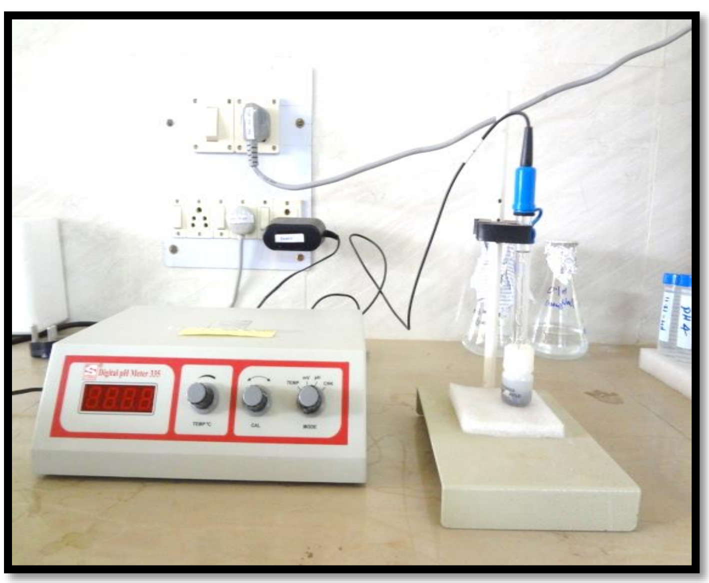

Fig. 3.18 pH meter used in the present study

Determination of particle size analysis

The percentage of various sizes of particles in a given dry sample is found by the mechanical analysis which performed in two stages, i.e. sieve analysis and hydrometer analysis as per IS: 2720 (Part - 4) (1985).

Determination of specific gravity

The specific gravity experiment for red mud, bio-neutralized red mud is done in pycnometer method as per IS: 2720 (Part - 3) (1980) and Le- Chatelier's apparatus was used for biopolymer modified red mud.

Consistency Limits Analysis

The values of liquid limit, plastic limit and plasticity index help in classifying the red mud, bio-neutralized red mud and biopolymer modified red mud was determined following IS: 2720 (Part-5) (1985).

Determination of Compaction characteristics

Compaction test determine the moisture content and dry density relationship as per IS: 2720 (Part-5) (1980) conducting two types of compaction i.e. (i) light compaction and (ii) heavy compaction. Here light compaction is done for red mud, biopolymer modified red mud and mini compacter is used for bio-neutralized red mud. Mini compacter was fabricated at NIT Rourkela following Sridharan and Sivapullaiah (2005) and calibrated with standard compaction. It is used throughout in compacting the bio-neutralized red mud as sample quantity was less. Details of mini compacter is explained in a brief below as follows.

Mini compacter

The sample mold is of 3.81cm internal diameter and 4.61cm external diameter and 10 cm in height. The sample mold assembly has a detachable base plate and a removable collar 3.50 cm height. The hammer assembly consists of a guiding frame and drop weight. The guiding frame consists of three detachable portions: top, middle, and bottom as shown in Figure 3.19. The guiding frame is such that the dropping weight is a floating weight between the bottom of the top portion and top of the bottom portion. The vertical rod in the middle portion acts as a bore guide for the hammer. The middle portion of the guiding rod, which is screwed to the top and bottom portion of the guiding assembly, can be detached for changing dropping weight for light (Proctor) and heavy compaction (modified Proctor) tests. The dropping weight is 1 kg for Proctor test and 2.5 kg for modified Proctor test. The hammers are 3.5 cm in height and 7-cm and 11-cm diameter for Proctor and modified Proctor, respectively, with a central bore of 2.0 cm, and fall freely through a height of 16 cm over the energy transferring foot.

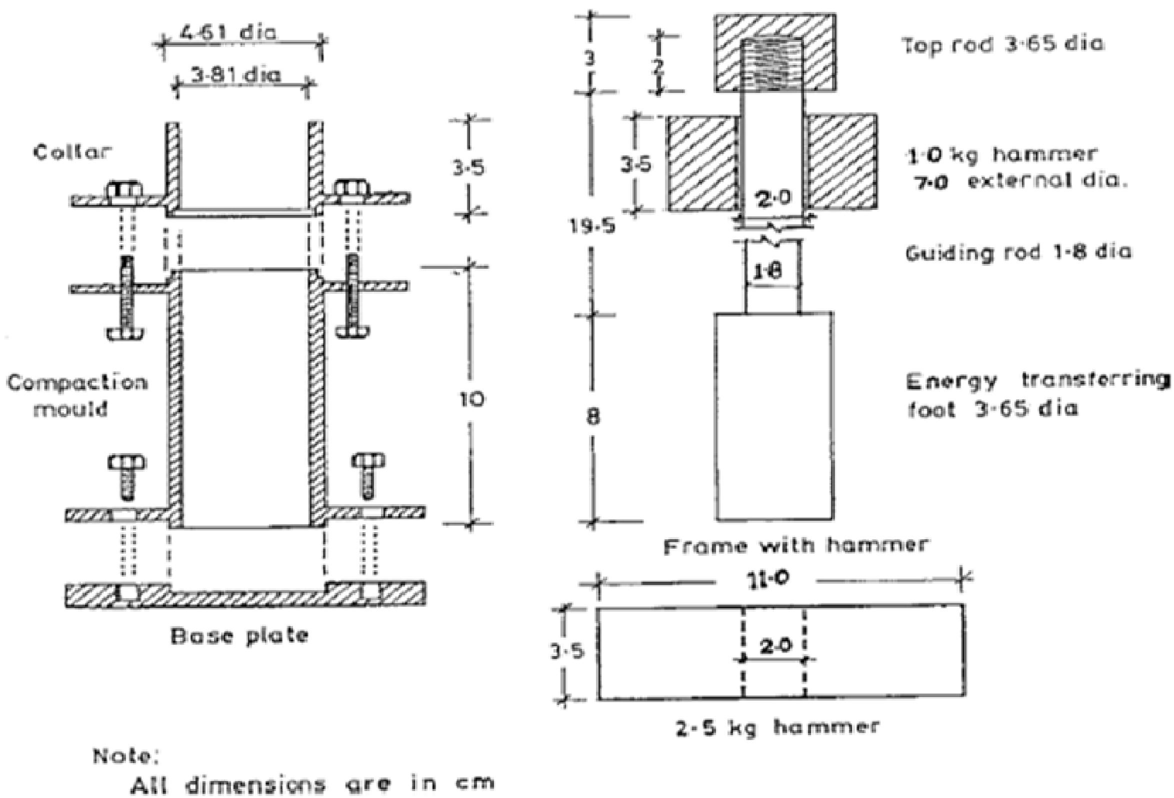

Fig. 3.19 Dimensions of mini mould (Sridharan and Sivapullaiah, 2005)

Procedure

Procedure remains same as for standard compaction test. Here for each compaction test about 300g of soil sample was used. The mold was cleaned, dried, and greased lightly to reduce the sidewall friction and for easy extrusion of compacted sample after the test and fixed to the base plate. The mold with the base plate was placed on a rigid platform. The soil was compacted in the mold in three layers. Approximate quantity of the soil required for the first layer was put in the mold, and then the required number of blows is applied to the soil by dropping the selected hammer on the energy transferring foot of the frame. Care was taken when the hammer stroke, the energy transferring foot that the frame (top rod) should not be in contact with the hand. After the required number of blows was applied, the soil surface was scarified before second layer was placed. The mold was filled with the soil for the second layer and again compacted. After the compaction of second layer, top collar was positioned to the mold and the third layer was placed and compacted. The compacted third layer should project above the top of the mold into the collar by not more than 5 mm. After the compaction, the collar was removed and excess soil was trimmed off to make even with the top of the mold. The weight of the compacted soil together with the mold was measured and the weight of the compacted soil was determined. The bulk unit weight of the soil was computed from the weight of the compacted soil and the volume of the mold. Knowing the water content and bulk unit weight, the dry unit weight was calculated. Figure 3.20 shows the image of mini compacter.

Fig. 3.20 Mini compacter with the mould

Determination of permeability

The permeability of soil sample was determined by consolidation test and equation for determination of permeability is as follows.

$$k = c_v \, m_v \, \gamma_w$$

Determination of unconfined compressive strength (UCS)

The UCS test for of red mud, bio-neutralized red mud and biopolymer modified red mud was performed as per IS: 2720 (Part -10) (1991).

Dispersion test

The properties of material such as soils or any kind of waste, i.e., red mud, fly ash, slag etc. that get dispersed or wash away in water is called as dispersiveness and caused due to the non-plastic nature and inadequate interparticle attraction. This property cannot be identified by the standard laboratory index tests such as grain size analysis, specific gravity or Atterberg's limits and hence, other laboratory tests have been derived for this purpose. The other laboratory tests performed are the crumb test, double hydrometer test, the pinhole test, turbidity test, the test of dissolved salts in the water and SAR (sodium absorption ratio) based tests.

Here, crumb test and double hydrometer test was performed. In double hydrometer test the dispersion ratio is defined as the ratio of percentage finer than 0.005 mm diameter measured without any dispersing agent to that measured with dispersing agent in a hydrometer test, which expressed in percentage. Table 3.2 shows the classification of dispersive soils based on double hydrometer test as per Volk (1937).

Table 3.2 Classification of dispersive soils based on double hydrometer test (Volk, 1937).

Dispersion ratio (%)	**Classification**
<35	Non-dispersive
35-50	Modestly dispersive
50-75	Highly dispersive
>75	Extremely dispersive

3.3.1.8 Particle Morphology, chemistry and mineralogical tests

Scanning Electron Microscope

Scanning Electron Microscope (SEM) with Energy Dispersive X-ray (EDX) micro analyzer was used in the present study for morphological and particle chemistry analysis. The chemical and mineralogical characterization of red mud and bio-neutralized red mud was not only beneficial for knowing its composition, but also helps in its classification for its possible utilization as an engineering material before and after treatment. The particle shape was quantified by using image analysis and documented with micrographs. The SEM used in the present study is JEOL-JSM-6480 LV model. The SEM is used to scan a finely focused beam of kilovolt energy. An image is formed by scanning electrode ray tube in synchronism with the beam and modulating the brightness of this tube with beam excited signals. The samples were prepared with carbon coating before being putting in the SEM. Figure 3.21 shows the layout of SEM set up with EDX microanalyses.

Fig. 3.21 SEM model JEOL JSM-6480LV for SEM and EDX analysis, NIT Rourkela

X-Ray Diffraction Analysis

X-ray diffraction (XRD) method used to carry out on the samples for qualitative identification of the mineral phases and quantitative estimates of mineralogical composition using Rietveld refinement methods for the red mud and bio-neutralized red mud samples. The powdered form of red mud samples were dried up at 105°C – 110^{o}C for 24 hours for X-ray diffraction analysis. The sample was analyzed by passing through a Philips diffractometer with a Cu Kα radiation source and a single crystal graphite monochromatic. An angular range of 10–70° of 2θ value (where θ is the incident/glancing angle of X-ray beam) in 0.1° increments was used throughout the experiment. Figure 3.22 shows the XRD assembly used in the present study.

Fig. 3.22 XRD model RIGAKU JAPAN/ULTIMA-IV for the mineralogical analysis

Chapter 4

Bio-neutralization of red mud

4.1 Introduction

The results of basic material properties of red mud and bio-neutralized red mud are discussed in this chapter. The main aim is to neutralize the red mud so as to reduce its alkalinity to be used as fill and pavement material. Therefore, the basic properties like chemistry, mineralogy, morphology etc. of red mud are analyzed before and after treatment for better characterization. In this chapter, results of methods of treatment of red mud followed by study of morphology, geotechnical properties etc. of treated red mud are presented. The above properties of bio-neutralized red mud are compared to that of raw red mud and analyzed.

4.2 Isolation of microorganisms from red mud (serial dilution process)

The procedure as described in Chapter 3 after isolation four different colonies were formed in 10^{-5} petri plate after 48 hours. The colonies formed were isolated for pure culture.

4.3 Streak plate method

The four bacterial colonies were grown after streaking method. It was named as RM 1, RM 2, RM 3, and RM 4 as described in Chapter 3. Figure 4.1 (a, b, c and d) shows the picture of four colonies isolated from red mud. All the cultures were added to red mud and pH reducing strains were identified.

It was observed that RM 1 had four different colored and different sized colonies, RM 2 had two different colonies, RM 3 had two different colonies and RM 4 had a single colony formed. Similarly, streaking was done again to obtain the pure culture from the above four plates along with three *Xanthomonas campestris* species obtained from NCIM, Pune repeating the same process. Figure 4.2 (a, b, c, d, e, f, g, h and i) shows pure cultured strains from isolated species of red mud and Figure 4.3 (a, b and c), shows three *Xanthomonas campestris* species

Fig. 4.1 (a) RM 1

Fig. 4.1 (b) RM 2

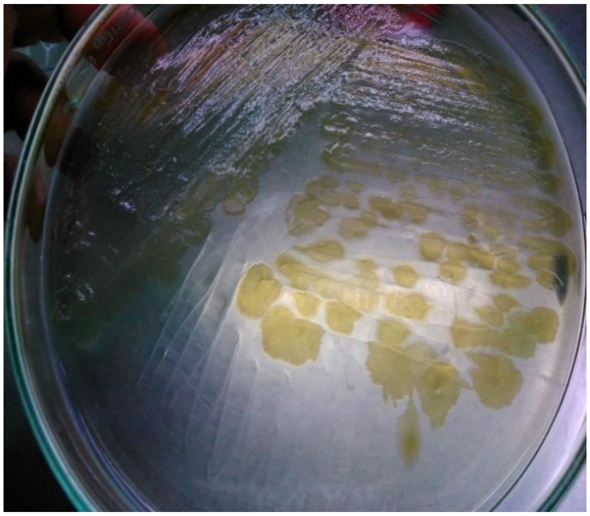

Fig. 4.1 (c) RM 3

Fig.4.1 (d) RM 4

Fig. 4.1 Strains of red mud isolate named as (a) RM 1 (b) RM 2 (c) RM 3 (d) RM 4

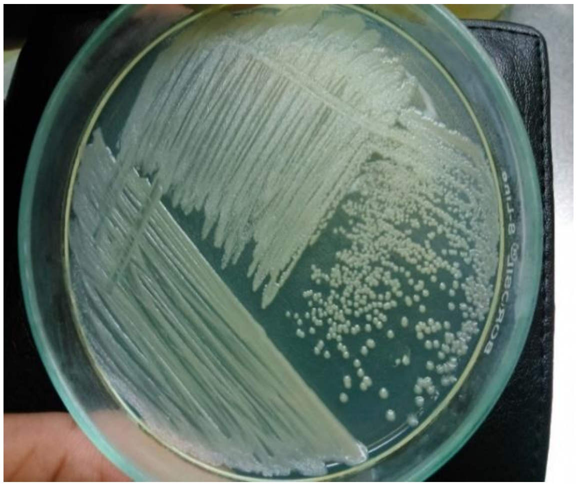

Fig. 4.2 (a) RM 1A

Fig. 4.2 (b) RM 1B

Fig. 4.2 (c) RM 1C

Fig. 4.2 (d) RM1 PA

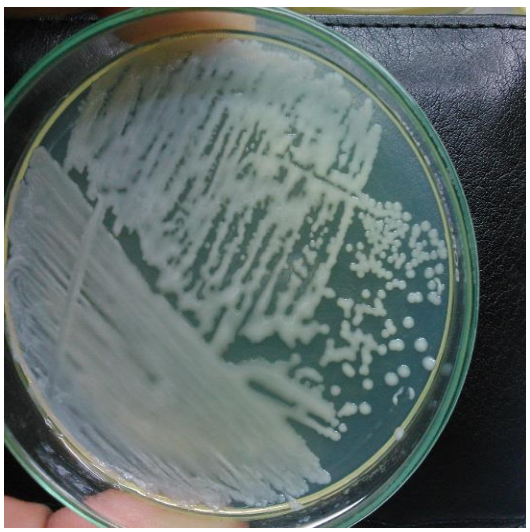

Fig. 4.2 (e) RM 1 PB

Fig. 4.2 (f) RM 2A

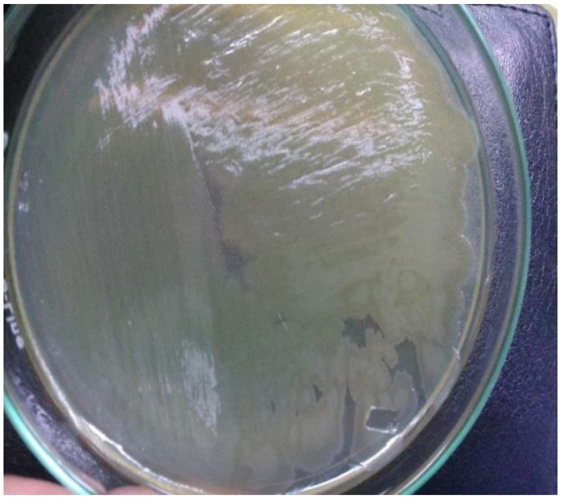

Fig. 4.2 (g) RM 3A

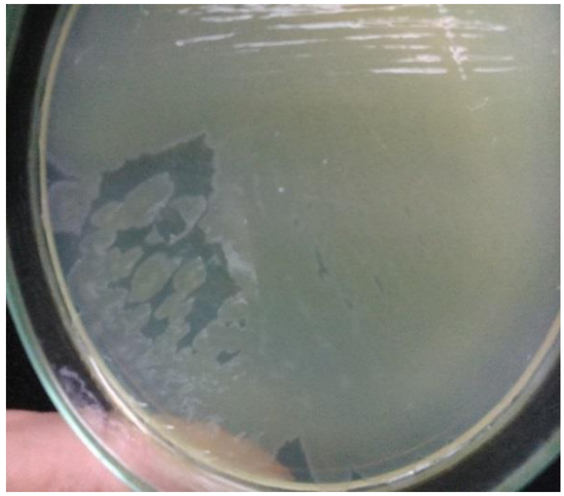

Fig. 4.2 (h) RM 3B

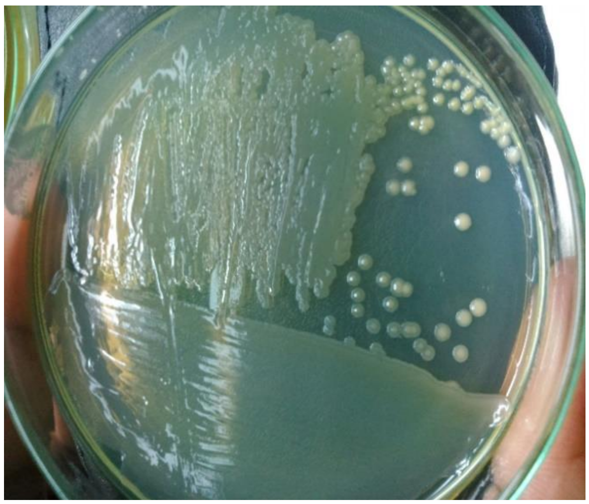

Fig. 4.2 (i) RM 4

Figure 4.2 Pure cultured strains from 12 isolated species named as (a**)** RM 1A (b**)** RM 1B (c) RM 1C (d) RM1 PA (e) RM 1 PB (f) RM 2A (g) RM 3A (h) RM 3B (i) RM 4

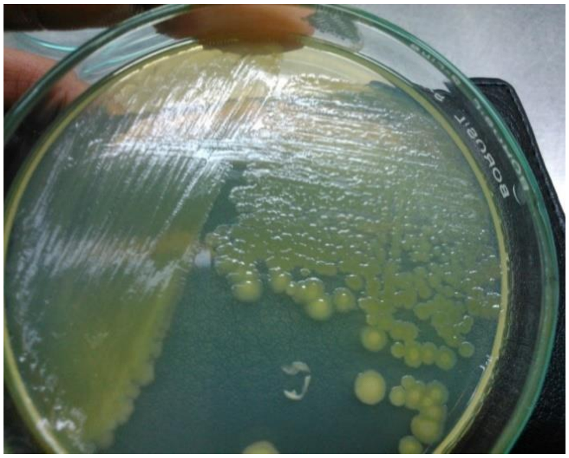

Fig. 4.3 (a) X Camp 2956

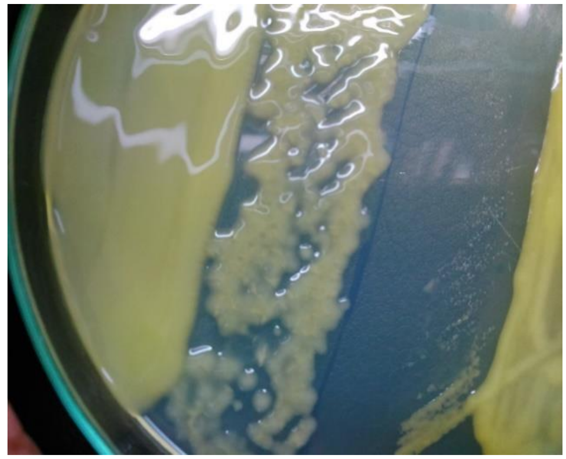

Fig. 4.3 (b) X Camp 2961

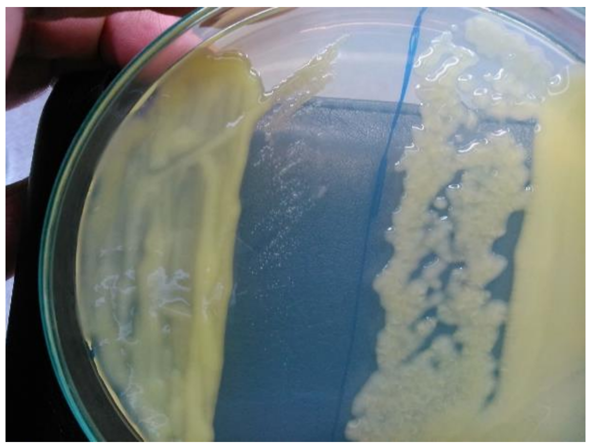

Fig. 4.3 (c) X Camp 5028

Figure 4.3 Xanthomonas campestris species (a) X Camp 2956 (b) X Camp 2961 (c) X Camp 5028

4.4 Nursery trials

Few trials were done to pick up the fastest pH reducing colonies. So all the colonies isolated along with a probiotic isolated by life science department, NIT Rourkela were inoculated with 15% red mud. Table 4.1 Initial and final pH values of bacteria with 15% red mud. It can be observed that fastest pH reducing colonies i.e. SAN 10, X camp 2956, RM 1C, RM 2A were picked up.

Table 4.1 Initial and final pH values of bacteria with 15% red mud

Sample	Initial pH	Final pH
MGYP + 15% RM (autoclaved control)	9.15	(1) 8.96 (2) 8.68
MGYP + 15% RM (un-autoclaved)	9.15	(1) 8.38
SAN 10 + 15% RM	9.15	(1) 7.40 (2) 7.36
X. camp 2956+ 15% RM	9.15	(1) 7.51 (2) 7.62
X. camp 5028+ 15% RM	9.15	(1) 8.58 (2) 8.48
SAE 13+ 15% RM	9.15	(1) 8.52 (2) 8.57
RM 4+ 15% RM	9.15	(1) 8.63 (2) 8.36
RM 3A+ 15% RM	9.15	(1) 8.36 (2) 8.40
RM 1B+ 15% RM	9.15	(1) 8.52 (2) 8.43
RM 3B+ 15% RM	9.15	(1) 8.45 (2) 7.75
RM 1A+ 15% RM	9.15	(1) 8.56 (2) 8.54
RM 1C+ 15% RM	9.15	(1) 7.47 (2) 7.40
X. camp 2961+ 15% RM	9.15	(1) 8.50 (2) 8.52
RM1 PB+ 15% RM	9.15	(1) 8.62 (2) 8.58
RM1 PA+ 15% RM	9.15	(1) 8.46 (2) 7.42
RM 2A+ 15% RM	9.15	(1) 7.59 (2) 7.55

In second attempt two new media composing of cheese whey and rice water were formulated and all pH reducing strains were inoculated with 30% red mud. Table 4.2 shows the initial and final pH values of bacteria with 30% red mud in cheese whey and rice water formulated media. It is observed that there is no change in pH of red mud.

Table 4.2 Initial and final pH values of bacteria with 30% red mud in cheese whey and rice water

Medium	Initial pH	Bacterial Name	pH
Cheese whey + red mud (30%)	9.63	RM 2A	(1) 9.10 (2) 9.05
Cheese whey + red mud (30%)	9.63	RM 1C	(1) 9.17 (2) 9.11
Cheese whey + red mud (30%)	9.63	SAN 10	(1) 9.13 (2) 9.12
Cheese whey + red mud (30%)	9.63	X. camp 2956	(1) 9.15 (2) 9.13
Cheese whey + red mud (30%)	9.63	Control	(1) 9.12 (2) 9.36
Rice water + red mud (30%)	9.67	RM 2A	(1) 9.54 (2) 9.53
Rice water + red mud (30%)	9.67	RM 1C	(1) 9.54 (2) 9.55
Rice water + red mud (30%)	9.67	SAN 10	(1) 9.55 (2) 9.54
Rice water + red mud (30%)	9.67	X. camp 2956	(1) 9.58 (2) 9.55
Rice water + red mud (30%)	9.67	Control	(1) 9.62

In third attempt two new media was formulated using both cheese whey and rice water and another only rice water and all the pH reducing bacteria along with four lactobacillus strains were inoculated with and without 15% red mud. The amount of red mud was reduced to half to check whether the bacteria can survive in the newly formulated media composition. Table 4.3 shows the initial and final pH values of bacteria with 15 % red mud in cheese whey with rice water and rice water media. Again it was observed that there is no change in the pH of red mud nor in the media.

Table 4.3 Initial and final pH values of bacteria with 15 % red mud in cheese whey with rice water and rice water media.

Type	Medium	Initial pH	Final pH
Control	CW+RW	7.03	7.03
	RW	6.65	6.65
	CWRW+RM (15%)	9.46	9.46
	RW+RM (15%)	9.82	9.82

SAN 10	CW+RW	7.03	6.55
	RW	6.65	6.32
	CWRW+RM (15%)	9.46	9.34
	RW+RM (15%)	9.82	9.85
RM 2A	CW+RW	7.03	6.20
	RW	6.65	6.33
	CWRW+RM (15%)	9.46	9.26
	RW+RM (15%)	9.82	9.82
RM 1C	CW+RW	7.03	6.15
	RW	6.65	5.33
	CWRW+RM (15%)	9.46	9.30
	RW+RM (15%)	9.82	9.83
X. camp 2956	CW+RW	7.03	6.76
	RW	6.65	6.24
	CWRW+RM (15%)	9.46	9.22
	RW+RM (15%)	9.82	9.77
Lab 1	CW+RW	7.03	6.17
	RW	6.65	5.98
	CWRW+RM (15%)	9.46	9.33
	RW+RM (15%)	9.82	9.85
Lab 2	CW+RW	7.03	6.13
	RW	6.65	6.00
	CWRW+RM (15%)	9.46	9.31
	RW+RM (15%)	9.82	9.84
Lab 3	CW+RW	7.03	6.32
	RW	6.65	5.98
	CWRW+RM (15%)	9.46	9.32
	RW+RM (15%)	9.82	9.85
Lab 4	CW+RW	7.03	6.27
	RW	6.65	5.97
	CWRW+RM (15%)	9.46	9.30
	RW+RM (15%)	9.82	9.85

It showed that the bacteria did not find a proper utilization source for their growth. Therefore in fourth attempt sugar molasses with cheese whey and rice water was added as they are a good source of carbohydrate utilization of bacteria. So again a new media was formulated constituting of sugar molasses, cheese whey and rice water. All the pH reducing strains were inoculated along with two probiotics and four lactobacillus strains were added without and with 15% and 30% red mud. Table 4.4 shows the initial and final pH values of bacteria without and with 15 % and 30%

red mud. From the table it is observed that bacteria can produce acid utilizing the carbon source in the production media and not in media with red mud.

Table 4.4 Initial and final pH values of bacteria without and with 15 % and 30% red mud

Type	**Medium**	**Initial pH**	**Final pH**
Control	SM+ Media	7.0	7.0
	SM+15% RM	9.8	9.8
	SM+30% RM	9.8	9.8
SAN 10	SM+ Media	7.0	(1) 6.08 (2) 6.06
	SM+15% RM	9.8	(1) 9.13 (2) 9.10
	SM+30% RM	9.8	(1) 9.69 (2) 9.13
RM 2A	SM+ Media	7.0	(1) 6.05 (2) 5.98
	SM+15% RM	9.8	(1) 8.82 (2) 7.24
	SM+30% RM	9.8	(1) 9.74 (2) 9.72
RM 1C	SM+ Media	7.0	(1) 6.15 (2) 6.10
	SM+15% RM	9.8	(1) 6.81 (2) 9.17
	SM+30% RM	9.8	(1) 9.72 (2) 9.70
X. camp 2956	SM+ Media	7.0	(1) 6.05 (2) 5.91
	SM+15% RM	9.8	(1) 7.05 (2) -
	SM+30% RM	9.8	(1) 9.36 (2) 9.67

The same procedure was repeated again taking without and with 15 % red mud. A new probiotic (SAE 33) was added this time. Table 4.5 shows the initial and final pH values of bacteria without and with 15 % red mud. From the table it is concluded that bacteria produces organic acid in its production media. So red mud was added after bacteria utilizes the carbon source present in media and produces acid and reduces the pH of media.

Table 4.5 Initial and final pH values of bacteria without and with 15 % red mud

Type	Medium	Initial pH	Final pH (1)	Final pH (2)	Final pH (3)
Control	SM	7.0	7.0	-	-
	SM+RM (15%)	9.8	9.8	-	-
RM 2A	SM	7.0	5.75	5.73	5.80
	SM+RM (15%)	9.8	9.86	9.81	9.84
RM 1C	SM	7.0	5.72	5.77	5.70
	SM+RM (15%)	9.8	9.84	9.86	9.79
X camp 2956	SM	7.0	5.73	5.73	5.69
	SM+RM (15%)	9.8	9.88	9.85	9.83
LAB 1	SM	7.0	6.34	6.14	6.33
	SM+RM (15%)	9.8	9.82	9.86	9.81
LAB 2	SM	7.0	5.62	5.74	5.67
	SM+RM (15%)	9.8	9.87	9.81	9.74
LAB 3	SM	7.0	6.15	6.13	6.12
	SM+RM (15%)	9.8	9.73	9.87	8.39
LAB 4	SM	7.0	6.11	5.82	6.30
	SM+RM (15%)	9.8	9.85	9.84	9.86
SAE 33	SM	7.0	5.82	5.74	5.80
	SM+RM (15%)	9.8	9.86	9.87	9.70
SAE 13	SM	7.0	5.27	5.12	-
	SM+RM (15%)	9.8	9.88	9.85	-

4.5 Morphology study

The pure culture obtained is studied with respect to their structural form. Morphological study is done on two pH reducing red mud isolates (RM 1C and RM 2A) which is used for bio-neutralization by staining procedure as described in Section 3.3.1.5. The result shows that RM 2A is pink in color and rod shaped, hence it is Gram negative. RM 1C is violet and rod shaped, hence Gram positive. The bacterial structure of RM 1C and RM 2A in 40X microscope is shown in Figure 4.4 and 4.5, respectively.

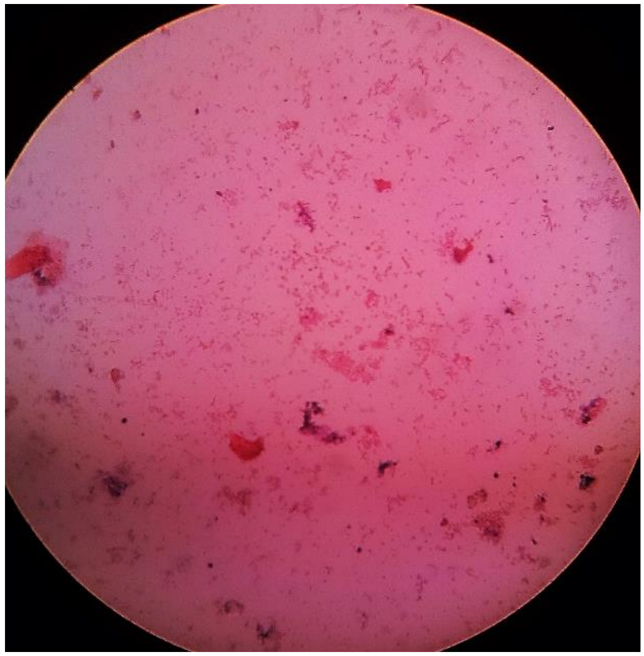

Fig. 4.4 Bacterial structure of RM 2A at 40X

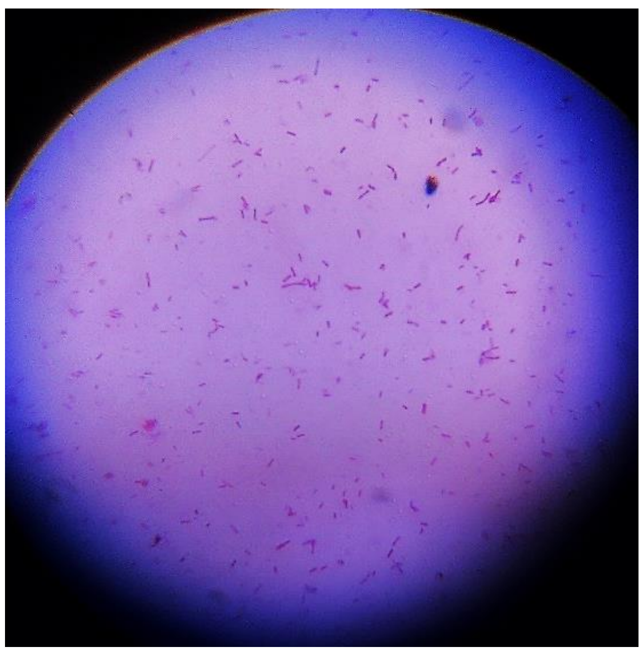

Fig. 4.5 Bacterial structure of RM 1C at 40X

4.6 Bio-neutralization process

The procedure used as described in the Section 3.3.1.4 was used to formulate the media for each strain and all the pH reducing strains were added to it. After 8-10 days the media's pH was checked again and 15% of red mud was added to each flask and kept overnight for shaking. After 24 hours, the media was taken out and pH was checked once again. The pH was found out to be around 7.5 which is optimal. The variance of pH of red mud is described below to counter check whether the media's pH has reduced or red mud's pH has reduced, so this treated red mud was kept for drying. After drying pH was again checked with distilled water with a known pH and compared with raw red mud which has a pH of 10.53. The variance of pH of sugar molasses media is given below Figure 4.6.

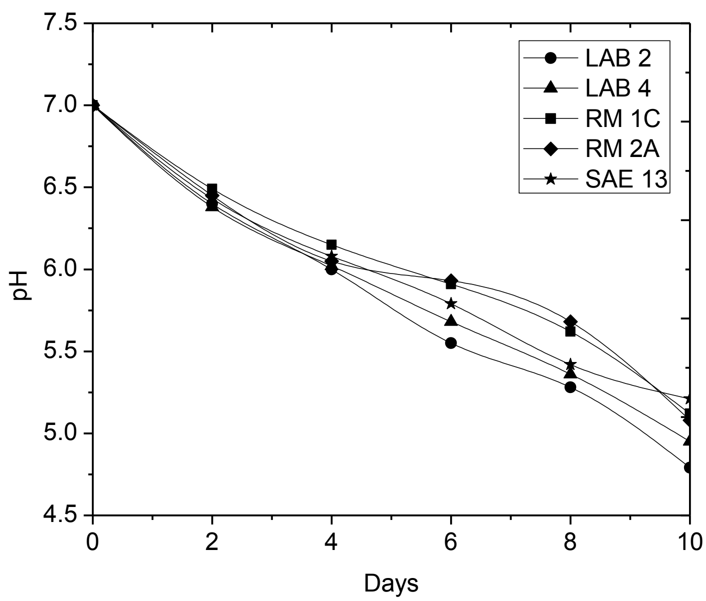

Fig. 4.6 pH variation curve of sugar molasses media

4.7 Total carbohydrate estimation

Total carbohydrate content was measured by anthrone method as described in Section 3.3.1.6. Carbohydrate estimation of bacterial growth media was done for initial day and final day i.e. after 10 days along with sugar molasses, cheese whey and rice water. Table 4.6 shows the total carbohydrate content of media and food waste. From the table it is observed that the initial content was 60.27g/l and after 10 days there is a reduction in the carbohydrate content. So it shows that bacteria utilizes the carbohydrate present in media for its growth and media pH reduces due to production of organic acid by the strains. Similarly total carbohydrate content of sugar molasses, cheese whey and rice water used throughout the experiment was also found out.

Table 4.6 Total carbohydrate content of media and food waste

Sample	Total carbohydrates (g/l)
Initial (0^{th} day)	60.27
LAB 2	11.56
LAB 4	23.44
RM 1C	15.72
RM 2A	1.13
SAE 13	21.15
Sugar molasses	202.89
Cheese whey	0.76
Rice water	10.29

4.8 Study of geotechnical properties

The colour of the red mud is normally brown to reddish brown due to the presence of iron oxide. There is colour change in red mud after neutralisation because of presence of molasses. The colour changes from red to blackish brown for LAB 2, LAB 4 and SAE 13. There is no change in colour of treated soil by RM 1C & RM 2A strains.

4.8.1 Particle size analysis

Figure 4.7 shows the grain size analysis of red mud and Figure 4.8 shows the grain size analysis of treated red mud done as per IS: 2720 (Part 4) (1985) using both sieve analysis and hydrometer analysis. It is observed that silt size is more than clay size for all the samples of red mud with and without treatment. It is classified under (ML-CL) group as per IS 1498 (1970).

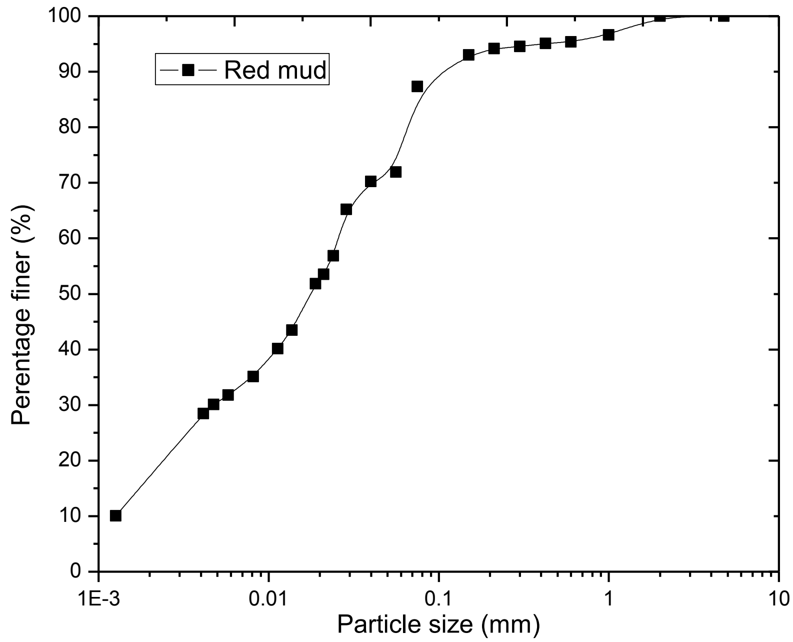

Fig 4.7 Grain size analysis of red mud

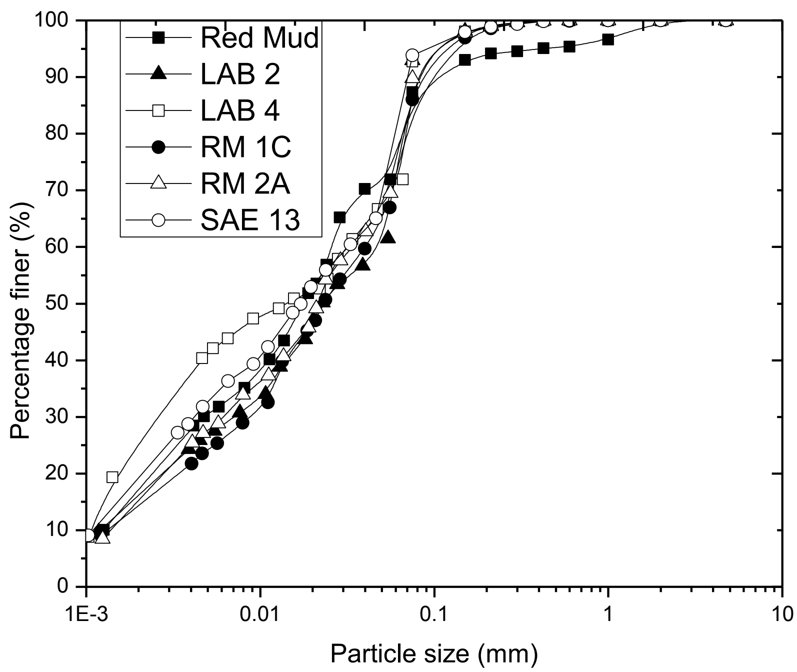

Fig 4.8 Grain size analysis of red mud and bio neutralized red mud

4.8.2 Specific Gravity

Specific gravity test was done for both red mud and bio-neutralized red mud. Table 4.7 shows the specific gravity of red mud & bio-neutralized red mud samples. It is observed that specific gravity varies with different strains. G_s value got reduced than ordinary red mud except for SAE 13 which has a G_s value of 3.45 same as raw red mud. Decrease in specific gravity value is due to the presence of organic compound produced by various bacterial strains inoculated.

Table 4.7 Specific Gravity of red mud & bio-neutralized red mud samples

Sample	Specific Gravity
RM	3.45
LAB 2	3.10
LAB 4	2.70
RM 1C	3.05
RM 2A	3.05
SAE 13	3.45

4.8.3 Atterberg Limits

Liquid limit (LL) and plasticity index (PI) of red mud and bio-neutralized red mud are evaluated. From the liquid limit and plasticity index red mud and LAB 2, LAB 4, RM 1C, RM 2A treated red mud is found to be inorganic silts of low plasticity (ML), and SAE 13 treated red mud is found to be inorganic clays of low plasticity (CL) as per Indian classification. Table 4.8 shows liquid limit, plastic limit, plasticity index of red mud and bio-neutralized red mud sample. From the table it is observed that the LL and PI value varies with different strains. There is a reduction in liquid limit (%) and plasticity index (%) as compared to normal red mud without treatment. The liquid limit and plasticity index is also reduced for treated red mud samples when it got neutralized by using sugar molasses. The low PI value may help in using treated as a filler material.

Table 4.8 Liquid Limit, Plastic Limit, Plasticity Index of red mud and bio-neutralized red mud sample

Sample	Liquid Limit (%)	Plastic Limit (%)	Plasticity Index (%)
RM	25.02	19.29	5.73
LAB 2	19.80	19.40	0.40
LAB 4	10.96	10.30	0.66
RM 1C	23.98	21.55	2.43
RM 2A	17.84	15.52	2.32
SAE 13	26.53	24.82	1.71

4.8.4 Compaction characteristics

The standard Proctor (light compaction) test result for red mud and bio-neutralized red mud is presented here. The maximum dry density (MDD) of red mud is found to be 18.37 kN/m^3 at light compaction with 22.6% of optimum moisture content. Fig. 4.9 shows the light weight compaction curve of red mud & bio-neutralized red mud. Table 4.9 shows the OMC and MDD of red mud bio-neutralized red mud samples. From the figures and table it is observed that the OMC and MDD value varies with different strains. There is a reduction in OMC (%) as compared to normal red mud without treatment and the MDD (kN/m^3) value remains nearly same for all the neutralized red mud samples. The reduction in OMC may be due to change in particle cohesion as reflected in reduction in PI value.

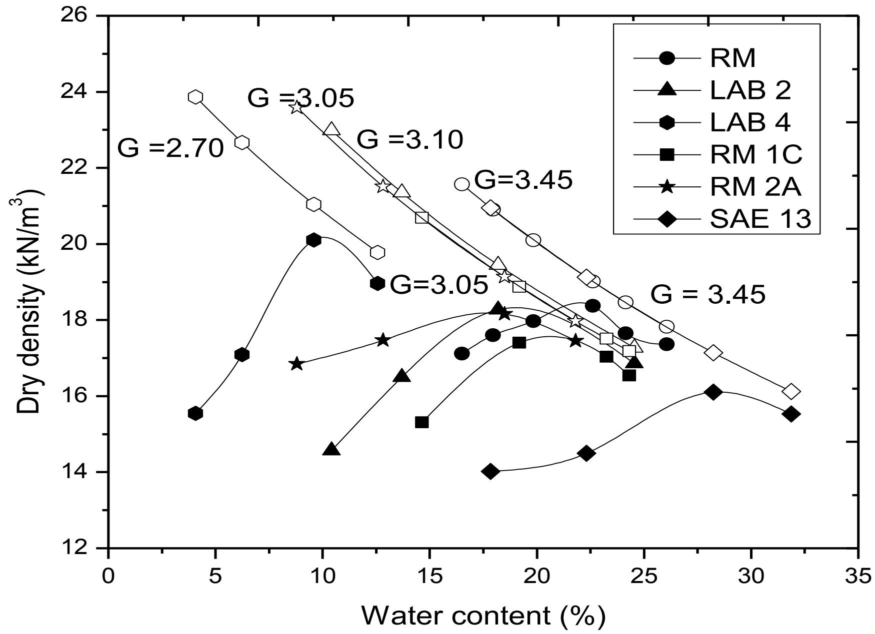

Fig. 4.9 Lightweight compaction curve of red mud & bio-neutralized red mud

Table 4.9 OMC and MDD of red mud bio-neutralized red mud samples

Sample	OMC (%)	MDD (kN/m^3)
RM	22.62	18.37
LAB 2	18.20	18.28
LAB 4	9.60	20.10
RM 1C	19.20	17.40
RM 2A	18.49	18.16
SAE 13	28.24	16.10

4.8.5 Unconfined Compressive Strength (UCS)

Keeping in mind, use of red mud as an embankment material, the shear strength of the red was obtained through unconfined compressive strength (UCS). Fig. 4.10 shows the stress vs strain curve of red mud & bio-neutralized red mud. Table 4.10 shows the UCS of red mud and bio-neutralized red mud samples. From the figure and table it is observed that UCS values remains almost same as compared to ordinary red mud for all the strains except for LAB 2, which has a higher UCS value of 298.6 kPa. It is also observed that LAB 4 has a low UCS value due to presence of sugar molasses which makes the soil soft and sticky. RM 1C and RM 2A has UCS values almost same and SAE 13 has higher UCS value as compared to ordinary red mud, which has a value of 136.5 kPa.

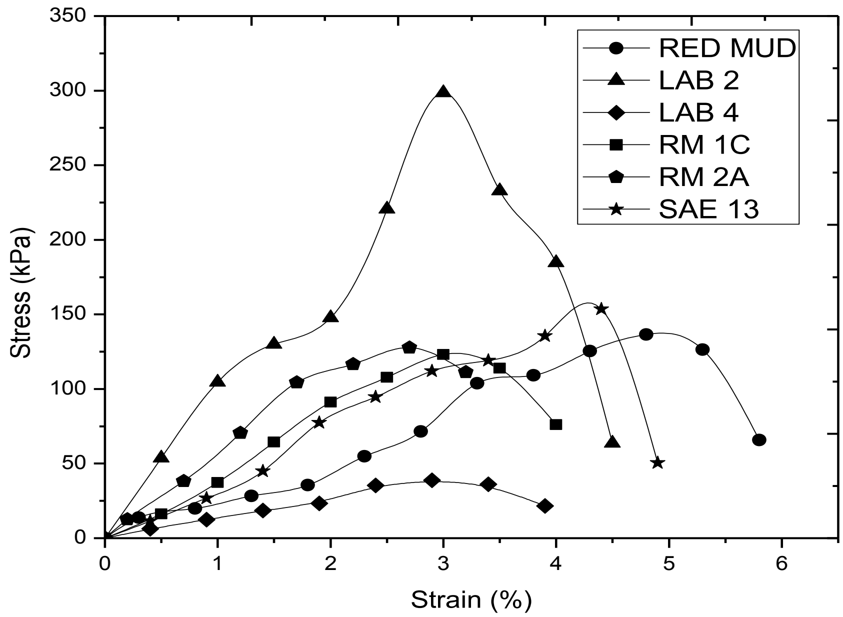

Fig. 4.10 Stress vs Strain curve of red mud & bio-neutralized red mud

Table 4.10 UCS of red mud and bio-neutralized red mud samples

Sample	Unconfined Compressive Strength (kPa)
RM	136.55
LAB 2	298.62
LAB 4	38.80
RM 1C	123.10
RM 2A	127.60
SAE 13	153.43

4.8.6 Co-efficient of permeability

The co-efficient of permeability test result for red mud and bio-neutralized red mud is presented here. Table 4.11 shows the co-efficient of permeability values of red mud bio-neutralized red mud samples. From the table it is observed that variation in co-efficient of permeability values of red mud bio-neutralized red mud samples are marginal compared to normal red mud samples.

Table 4.11 Co-efficient of permeability values of red mud bio-neutralized red mud samples

Sample	k (cm/sec)
RM	5.131×10^{-8}
LAB 2	1.23×10^{-8}
LAB 4	3.303×10^{-8}
RM 1C	8.972×10^{-9}
RM 2A	2.904×10^{-8}
SAE 13	4.108×10^{-8}

4.9 Particle Morphology, chemistry and mineralogical tests

4.9.1 Particle morphology

The micro morphology of materials is tested using Scanning Electron Microscope (SEM). The SEM micrograph of fine grain red mud at 5000 magnification is presented in Figure 4.11. It was observed that red mud contains very irregular spherical particles whereas treated red mud (LAB 2, LAB 4, RM 1C, RM 2A & SAE 13) at same magnification shown in Figure 4.12 to Figure 4.16 respectively, have agglomerated structure and may be due to binding of particles with the organic compound used.

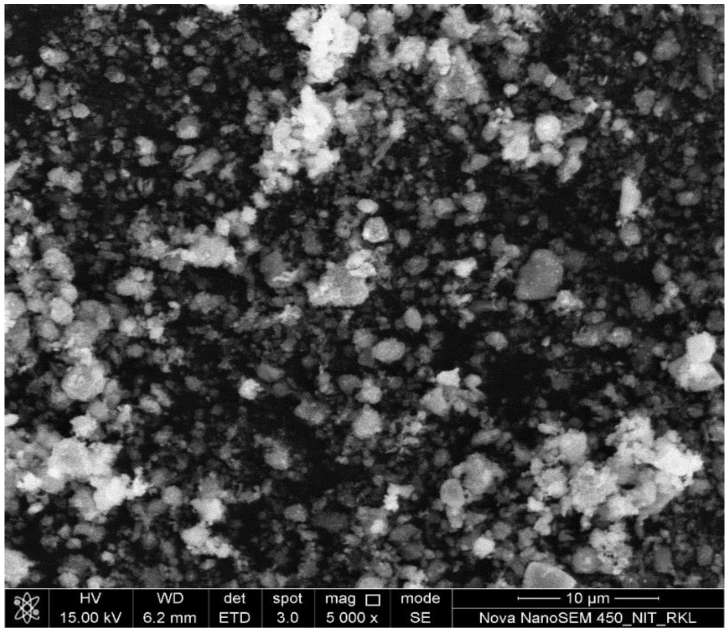

Fig. 4.11 Scanning electron micrograph of red mud at 5000 magnification.

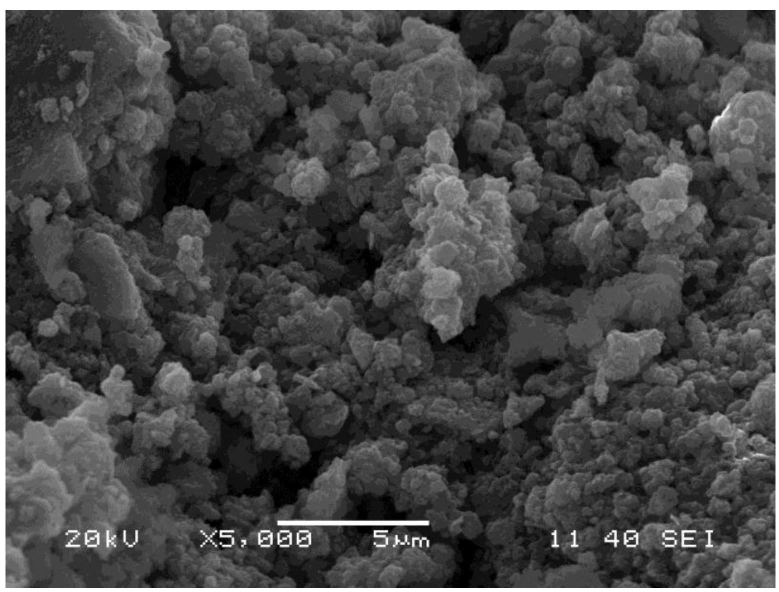

Fig. 4.12 Scanning electron micrograph of LAB 2 treated red mud at 5000 magnification.

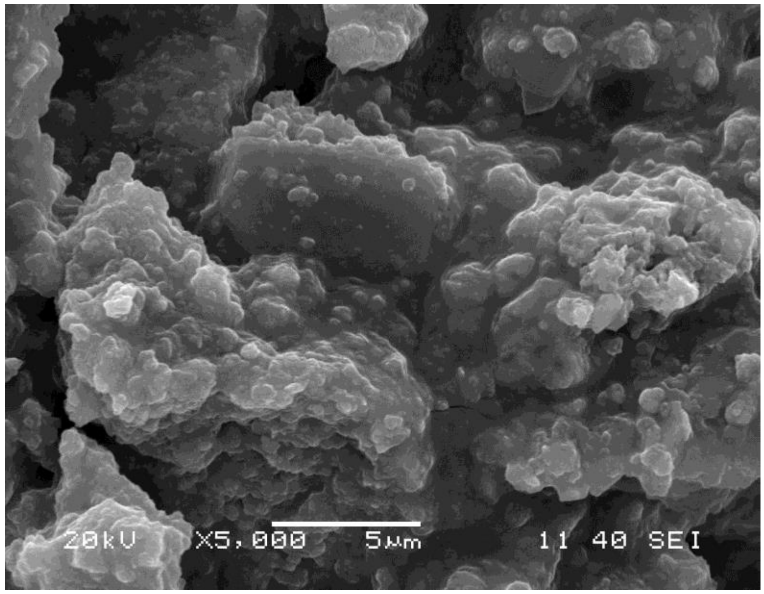

Fig. 4.13 Scanning electron micrograph of LAB 4 treated red mud at 5000 magnification.

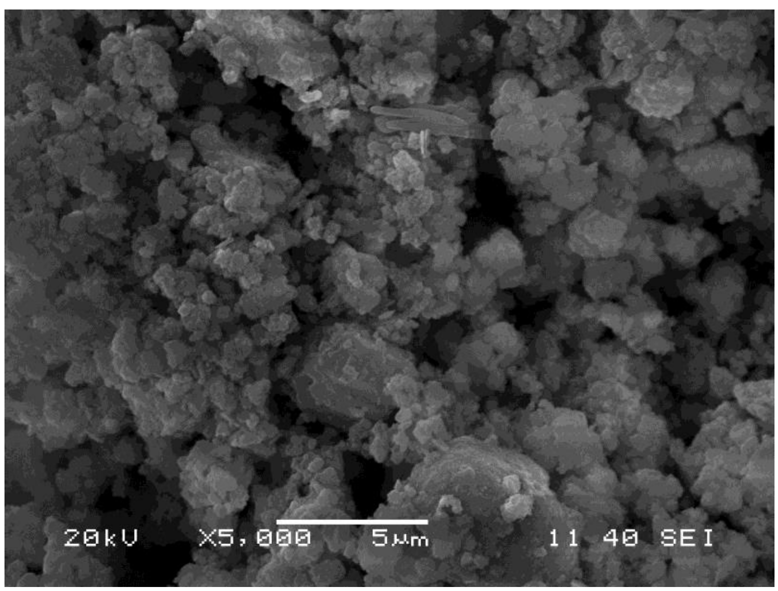

Fig. 4.14 Scanning electron micrograph of RM 1C treated red mud at 5000 magnification.

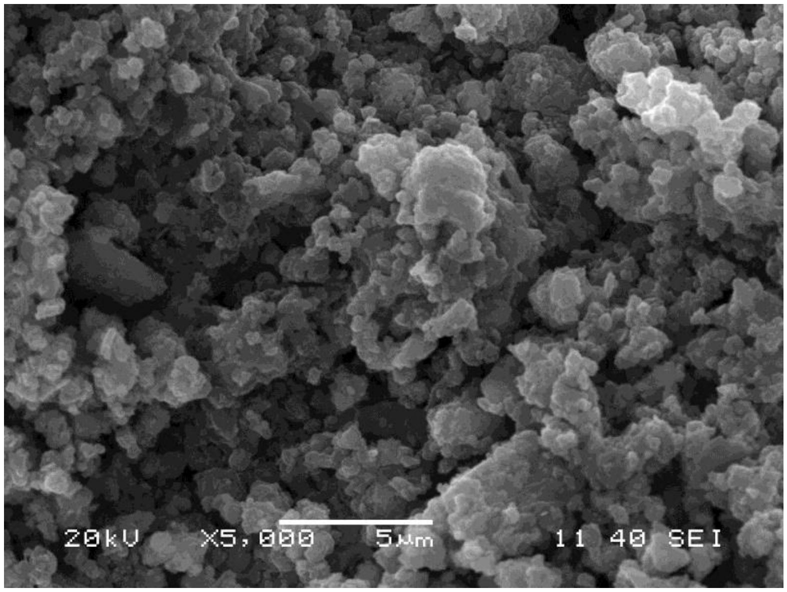

Fig. 4.15 Scanning electron micrograph of RM 2A treated red mud at 5000 magnification

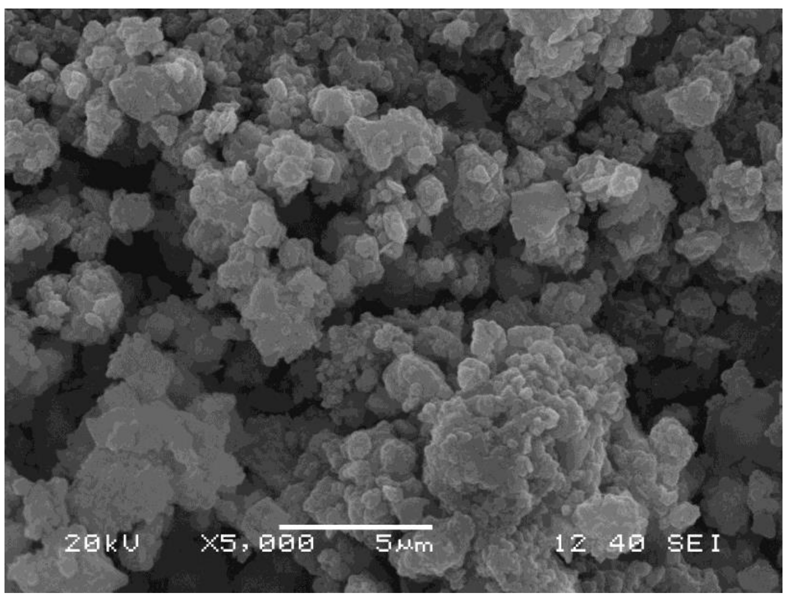

Fig. 4.16 Scanning electron micrograph of SAE 13 treated red mud at 5000 magnification

4.9.2 Mineralogical investigation

The XRD test results of red mud and bio-neutralized red mud sample are shown in figure below. From the figure 4.17 it can be observed that red mud has gibbsite, hematite, quartz and vanadium oxide are pre-dominantly present. Bio-neutralized red mud image shows that the intensity of gibbsite, hematite and quartz amount decreases in the treated red mud and new compound like cristobalite, tridymite was formed as shown in Figure 4.18 to Figure 4.22. The variation in mineral content may due to development, manifestation of new minerals with bioremediation. More tests are required to established the above result.

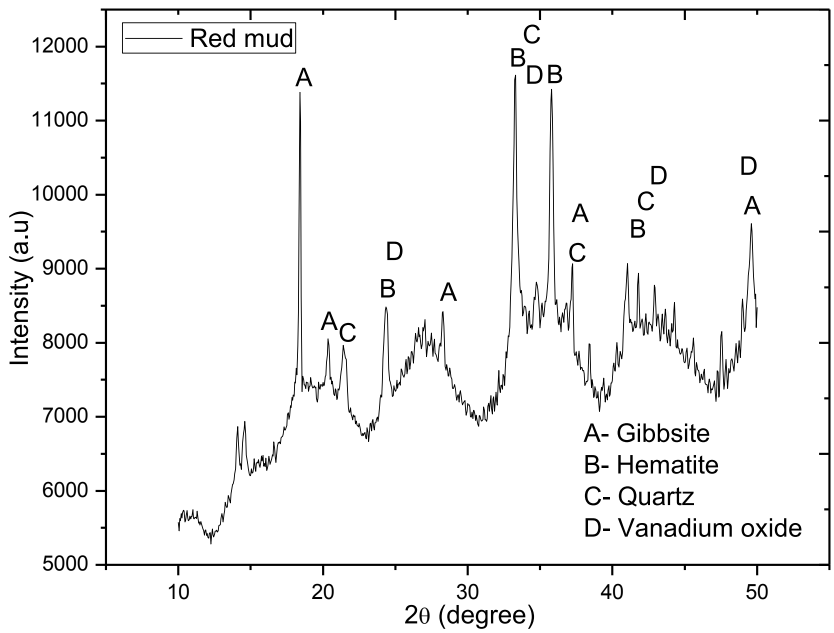

Fig. 4.17 XRD plot for red mud

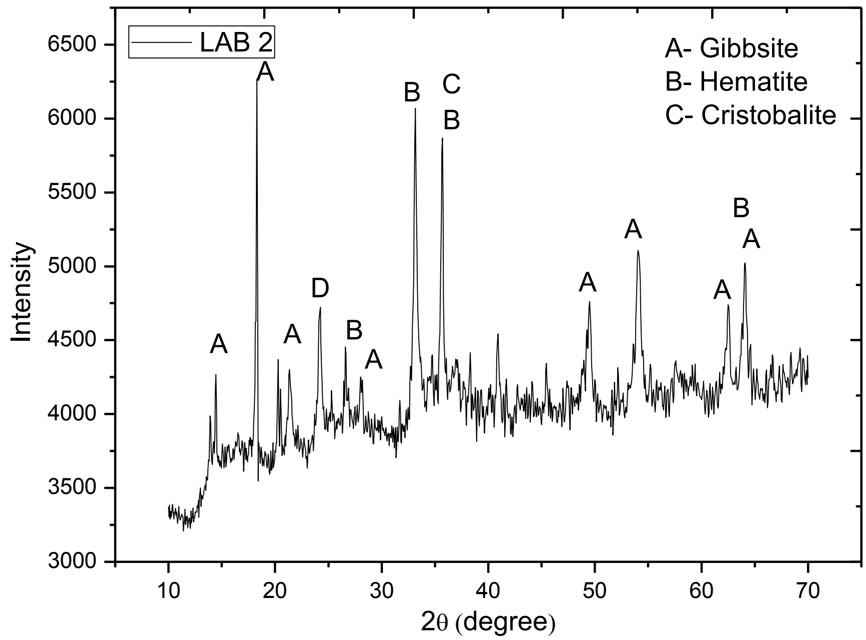

Fig. 4.18 XRD plot for LAB 2 treated red mud

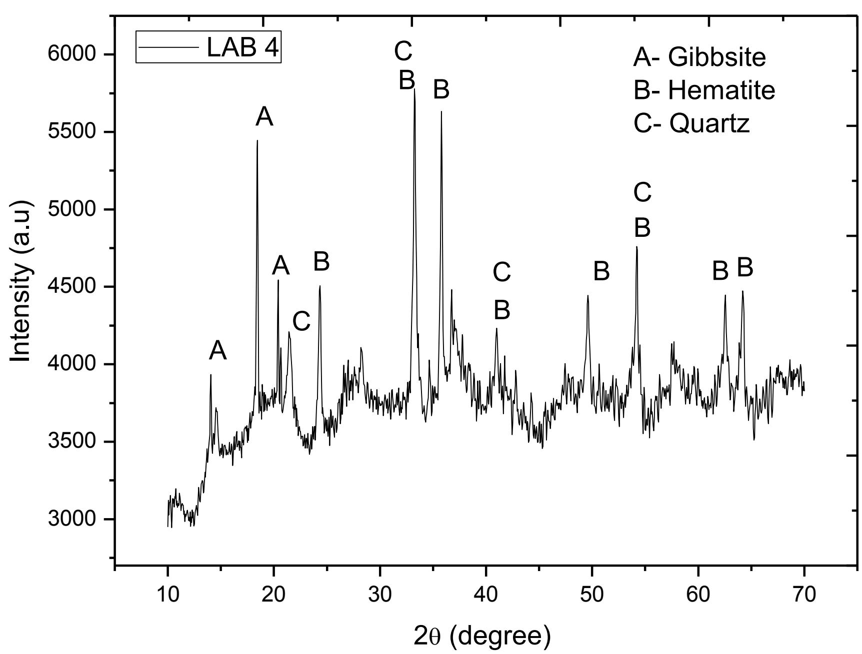

Fig. 4.19 XRD plot for LAB 4 treated red mud

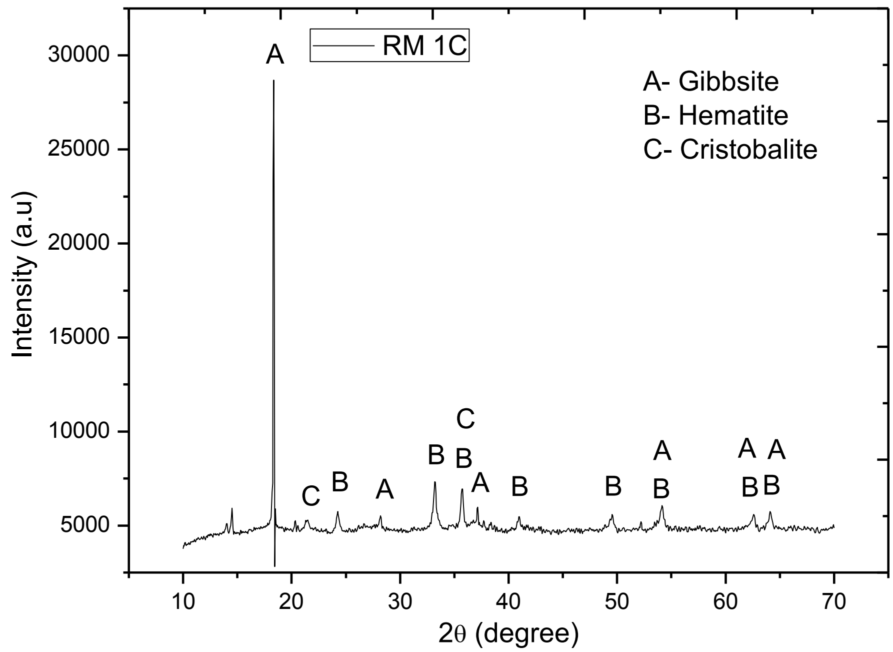

Fig. 4.20 XRD plot for RM 1C treated red mud

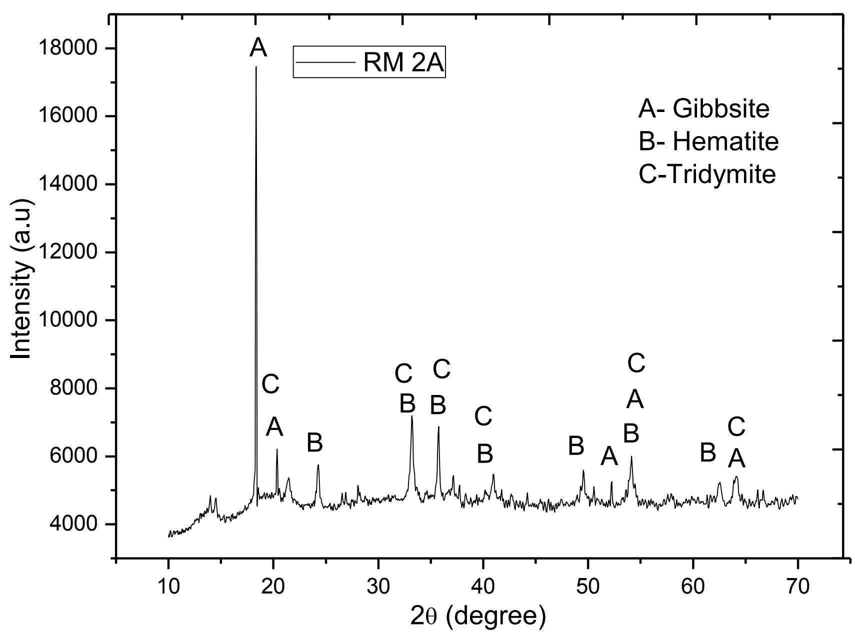

Fig. 4.21 XRD plot for RM 2A treated red mud

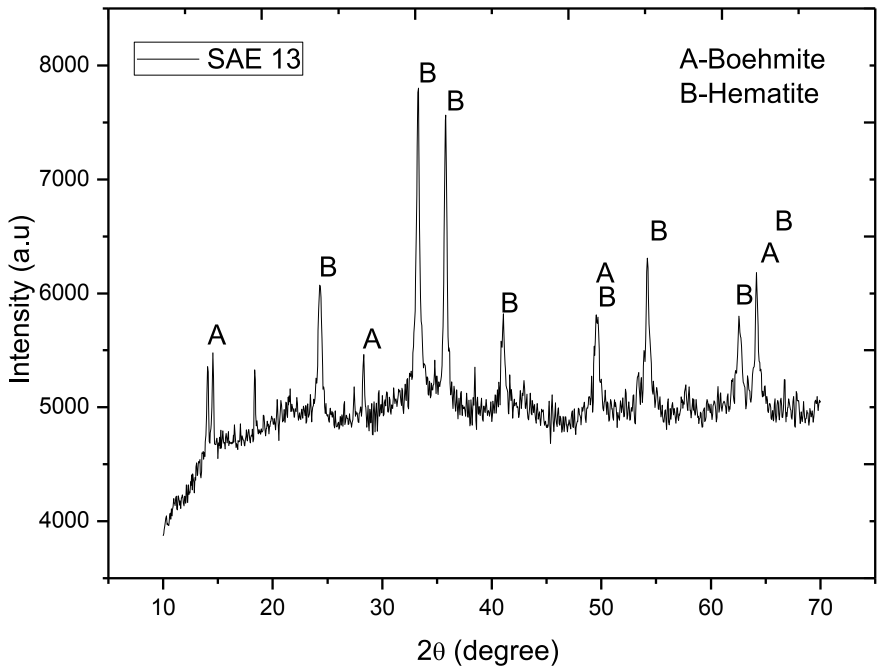

Fig. 4.22 XRD plot for SAE 13 treated red mud

Chapter 5

Stabilization of red mud using biopolymer

5.1 Introduction

This chapter presents the results of stabilization of red mud with biopolymer modified red mud concerning factors like dispersion, wind erosion, dust control and other environmental factors concerning in the dumping areas of red mud. The main aim is to stabilize the red mud with a biopolymer Xanthan gum. Compaction tests were done to obtain the optimum moisture content and maximum dry density and also unconfined compressive tests were conducted on samples of red mud without and with various percentage of Xanthan gum (1%, 2%, and 3%). The increase in strength criteria is determined by conducting unconfined compression test on samples at 0, 3, and 7 days curing. Similarly increase in strength criteria is also observed by keeping the biopolymer modified red mud without curing in sunlight to check the stability, concerning the above mentioned factors. It was observed that red mud mixed with various percentage of Xanthan gum have more strength than ordinary red mud samples. Results also indicated that the gum do not react with red mud reflecting it to be sustainable and effective to use.

5.2 Specific gravity

Specific gravity test was done for both the red mud and biopolymer stabilized red mud. It is observed that specific gravity of each sample when added with different percentage of bio polymer decreases as compared to the raw red mud. Table 5.1 shows the variation of specific gravity of red mud and bio polymerized red mud. This decrease in specific gravity may be due to the low specific gravity of Xanthan gum.

Table 5.1 Specific Gravity of red mud & bio polymer modified red mud samples

Soil Sample	Specific Gravity
Red Mud	3.45
RM+XG (1%)	3.25
RM+XG (2%)	3.11
RM+XG (3%)	3.11

5.3 Compaction characteristics

The following graphs shows the compaction characteristics of red mud and biopolymer modified red mud, showing optimum moisture content (OMC) and maximum dry density (MDD) of the compacted samples. Figure 5.1 shows lightweight compaction curve of red mud & biopolymer modified red mud. From table 5.2 it is observed that OMC increased with increase in gum percentage and higher than ordinary red mud which has an OMC of 22.62%. But MDD value decreased with higher gum percentage as compared to ordinary red mud. The loss in MDD is due to low specific gravity value of the mixture.

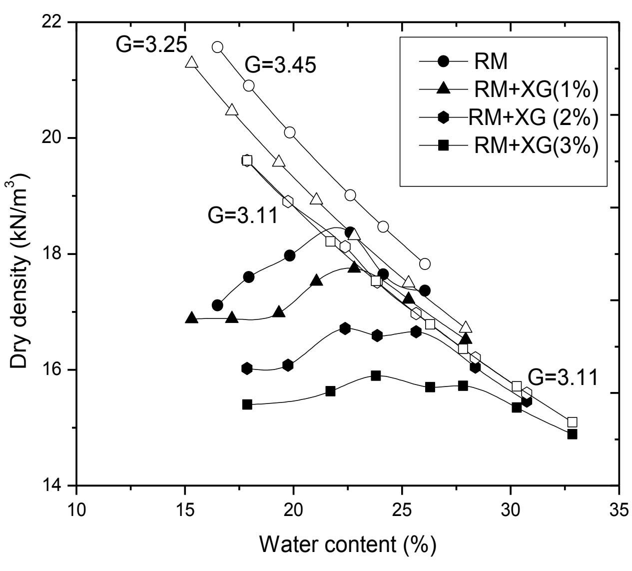

Fig. 5.1 Lightweight compaction curve of red mud & biopolymer modified red mud

Table 5.2 OMC & MDD values of red mud & biopolymer modified red mud samples

Soil Sample	OMC (%)	MDD (kN/m^3)
Red Mud	22.62	18.37
RM+XG (1%)	22.80	17.75
RM+XG (2%)	22.38	16.71
RM+XG (3%)	23.79	15.90

5.4 Unconfined Compressive Strength (UCS)

The UCS values of red mud and biopolymer modified red mud, showing stress of with and without cured samples are presented as follows. Figure 5.2 shows stress vs strain curve for 0^{th} day curing of red mud & biopolymer modified red mud. Similarly Figure 5.3, 5.4 and 5.5 show stress vs strain curve of red mud & biopolymer modified red mud for 3 day,7 day and sundried (3 days) curing. Table 5.3 shows UCS value of red mud and biopolymer modified red mud samples. It is observed that red mud mixed with Xanthan gum has more strength than ordinary red mud. Results illustrate that red mud with 1% Xanthan gum has more strength and shows optimum result at 1%. It was observed that with addition of XG more than 1%, the mixture became soft and sticky. Hence, 1% XG was found to be the optimum percentage.

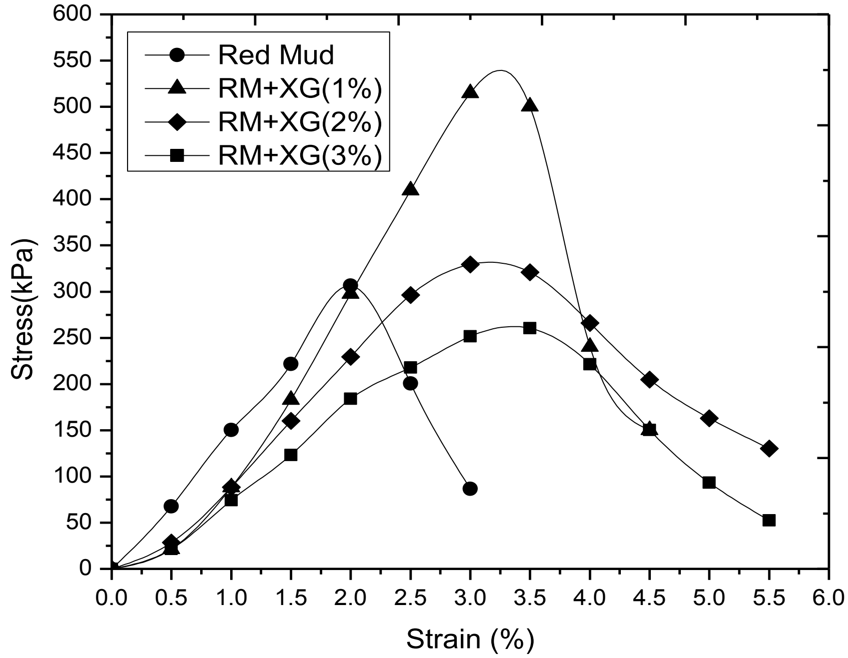

Fig. 5.2 Stress vs Strain curve for 0^{th} day curing of red mud & biopolymer modified red mud

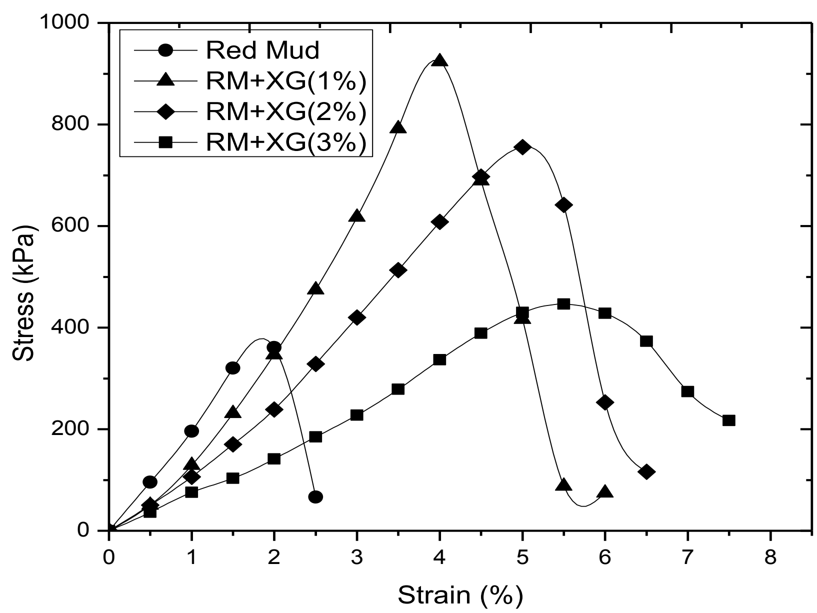

Fig. 5.3 Stress vs Strain curve for 3 days curing of red mud & biopolymer modified red mud

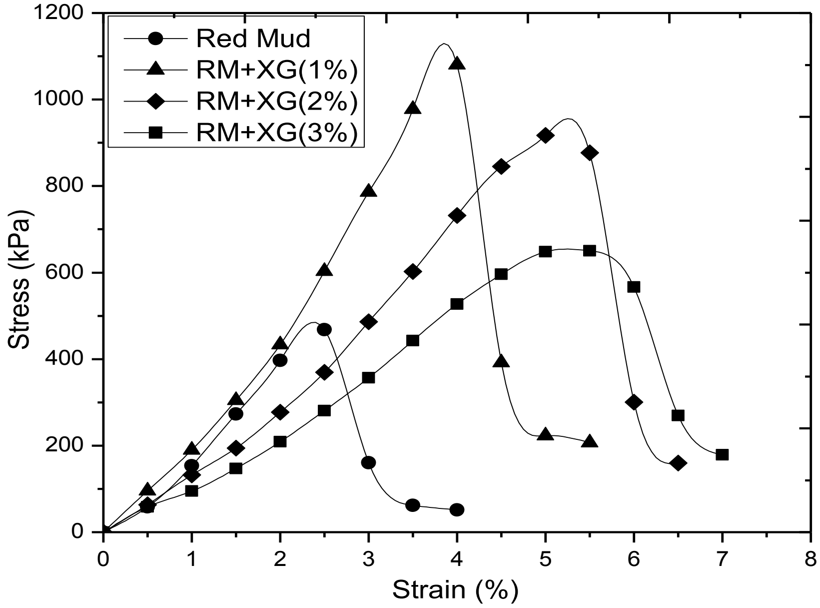

Fig. 5.4 Stress vs Strain curve for 7 days curing of red mud & biopolymer modified red mud

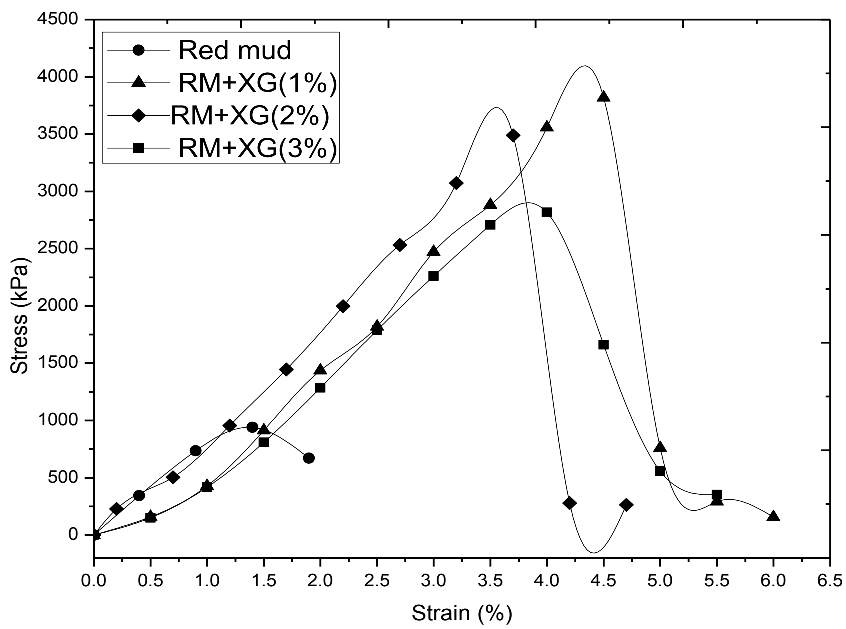

Fig. 5.5 Stress vs Strain curve for sundried (3 days curing) of red mud & biopolymer modified red mud

Table 5.3 UCS value of red mud and biopolymer modified red mud samples

Soil Sample	UCS (kPa)			
	0 day	**3 days**	**7 days**	**Sundried (3 days)**
Red Mud	306.59	360.48	467.97	940.74
RM+XG (1%)	525.01	923.56	1080.25	3819.76
RM+XG (2%)	329.47	446.54	917.00	3488.71
RM+XG (3%)	260.50	643.32	650.12	2815.82

5.5 Dispersion test

Dispersive behavior of red mud particles was studied using double hydrometer test and the dispersion ratio is found to be 88.7% which is highly dispersive as per Volk (1937) (Table 3.2) . Crumb test also conducted to know the dispersiveness. It is observed that red mud is more dispersive and miscible in water. But red mud added with various percentages biopolymer is less miscible due to bonding between soil particles. Figure 5.6 shows that red mud is dispersed away in 5-7 minutes whereas in Figures 5.7, 5.8 and 5.9 shows red mud with different percentages of gum (1%, 2% and 3%) respectively, is not dispersive in water and due to bonding between particles.

Fig. 5.6 Dispersion test on red mud

Fig. 5.7 Dispersion test on RM + XG (1%)

Fig. 5.8 Dispersion test on RM + XG (2%)

Fig. 5.9 Dispersion test on RM + XG (3%)

5.6 Particle Morphology, chemistry and mineralogical tests

5.6.1 Particle morphology

As shown in Figure 4.11 the SEM micrograph of fine grain red mud at 5000 magnification has irregular spherical particles. Figure 5.10 shows scanning electron micrograph of red mud with Xanthan gum at 5000 magnification. It is observed that particles are bind together and agglomerated particles were observed. This is due to binding of particles with Xanthan gum.

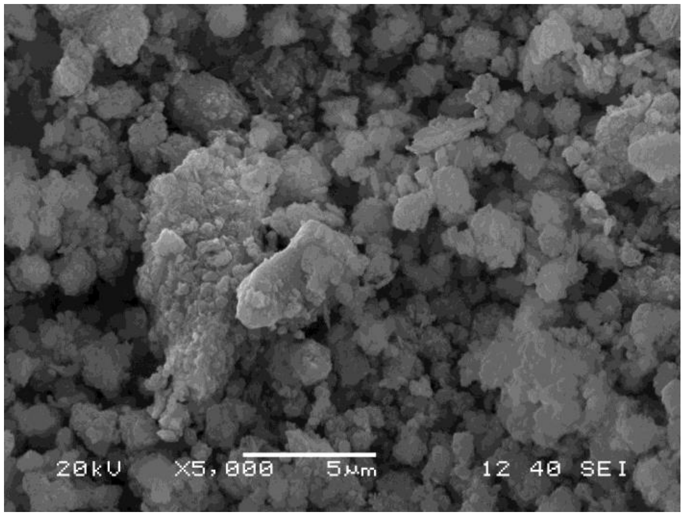

Fig. 5.10 Scanning electron micrograph of red mud with Xanthan gum at 5000 magnification

5.6.2 Mineralogical investigation

The XRD test results of biopolymer modified red mud sample are shown in Figure 5.11 below. It is observed that hematite, quartz and calcite are predominantly present as that of original red mud (Figure 4.17). It is due to the reason that there is no chemical interaction between red mud particles and Xanthan gum.

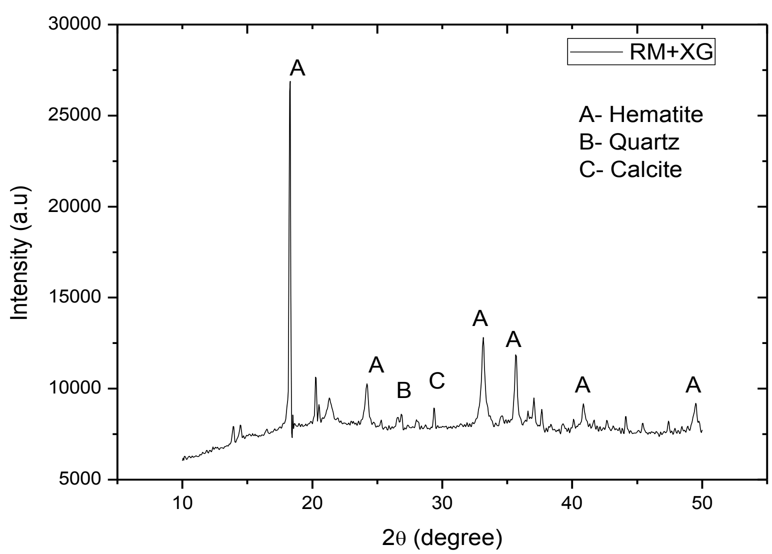

Fig. 5.11 XRD plot of red mud with Xanthan gum

Chapter 6

Conclusion and Future Scope

6.1 Summary

The first key issue that was identified in this project was the lack of availability and organization of the existing knowledge relating to management of bauxite residue. About 120 million tons of red mud is produced per year in aluminum industry over worldwide. Though it is utilized in various sectors worldwide but it only 10% of it can be utilized as construction material i.e. as embankments and fills and less than 5% of red mud is neutralized before using it as construction material. So the red mud is neutralized and stabilized in terms of soil as construction material. The present study concludes the laboratory tests like morphology, chemistry and geotechnical index properties of red mud and bio-neutralized red mud and biopolymer stabilized red mud. A comparison of some properties is made of red mud and bio-neutralized red mud and biopolymer stabilized red mud.

6.2 Conclusions

The following observations can be made based on the above from Chapter 1 to Chapter 5.

1. The alkalinity of red mud is high with a pH value 10.53 due to presence of NaOH and Na_2CO_3. This high pH value of red mud is one of the hindrances for its effective utilization and management.
2. In this study bacteria cultures (LAB 2, LAB 4, RM 1C, RM 2A and SAE 13) reduced the pH value of red mud to ~7.5 from 10.53, when red mud was mixed with various organic wastes as a cheap source for carbohydrate (food) of the bacteria.

3. As RM 1C and RM 2A are isolated from the red mud itself, hence these two methods are recommended for treatment of red mud to be used as geotechnical engineering material.
4. Though there was a reduction in OMC value of treated red mud, the MDD values remains same as compared to raw red mud. This may be due to reduction in specific gravity of the treated red mud.
5. As per IRC standards, plasticity index (PI) is considered as one of the important parameter with PI < 6 as the suitable for a material to be used as filler material and reduction in plasticity index shows that it can be used as filler material in pavements. Though, more tests like drainage, serviceability need to be done to draw final conclusions in this regard.
6. The red mud is extremely dispersive nature as determined in double hydrometer test with dispersion ratio of 88% and crumb test also gave similar results. This study also suggests the stabilization of red mud using biopolymer Xanthan gum can mitigate the dispersibility property of the soil in the stack areas, controlling the dust nuisance and wind erosion of red mud. By the use of biopolymer, Xanthan gum (1%, 2% and 3%) the red mud became non-dispersive.
7. Optimum moisture content of biopolymer modified red mud (1%, 2% and 3%) was slightly more than red mud and MDD decreased from 18.87 kN/m^3 to 15.9 kN/m^3.
8. Unconfined compressive results show that optimum result was obtained at 1% of Xantham gum and strength increases with days cured.

The present study will help in the safe disposal and management of red mud after bioremediation.

6.3 Future scope

There is a vast scope to use red mud in huge quantities as fill and embankment material. The neutralization and stabilization of red mud in this study is limited to a single source for geotechnical characterization and laboratory investigations. Some of the verdicts are recognized for future studies.

1. In-situ studies and its laboratory validation of properties of red mud from different sources and different storage times.
2. Stabilization of neutralized red mud using other methods of stabilization and using other soils to be used as a clay liner, fill material etc.
3. Screening and identification of other microorganisms in red mud neutralization.
4. Effect of other biopolymer in red mud stabilization.

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